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13. ABSTRACT ***(Maximum 200 Words)***

This entire project is based on the hypothesis that we can design and develop new synthetic triterpenoids that would eventually be useful for chemoprevention of prostate cancer. With the known importance of oxidative stress and the known involvement of the enzymes, inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), in the process of carcinogenesis in several other organs, and our own preliminary findings that new synthetic triterpenoids can block de novo induction and synthesis of both these enzymes, there is now a sound mechanistic basis for this hypothesis. Furthermore, since we have already shown that new synthetic triterpenoids can inhibit cell growth, without evident cytotoxicity, in non-malignant prostate epithelial cells, we believe that it will be possible to design and synthesize even more effective triterpenoids for this purpose. Finally, it is critical that a receptor (or receptors) for triterpenoids be defined, since these are presently unknown.

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FOREWORD

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N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Michael B. Spinn, M.D.

PI - Signature 12-06-13 Date

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Introduction

There is a major need for new drug discovery in the field of prostate cancer. There is a particular need for developing new agents to prevent this disease, since screening techniques are now identifying large numbers of men with early, pre-malignant lesions in their prostate. Such lesions do not require surgery and are not treatable with conventional chemotherapy. However, men with this type of pre-malignant condition are at definite risk for future development of invasive, metastatic prostate cancer which is life-threatening. This project has attempted to develop a new class of molecules, the triterpenoids, as chemopreventive agents which could eventually be used to prevent prostate cancer in men at high risk.

Body

Statement of Work:

Triterpenoids and Prevention of Prostate Cancer

Michael B. Sporn, Principal Investigator

Task 1: To synthesize new triterpenoids and test them as inhibitors of de novo synthesis of iNOS and COS-2
- Continue efforts to make new triterpenoid molecules
- Perform assays by Northern blot analysis to determine effects on transcription of iNOS and COX-2 genes
- Perform assays by Western blot analysis to determine effects on synthesis of new iNOS and COX-2 proteins.

Task 2: To evaluate new triterpenoids as inhibitors of growth of prostate cells
- Perform assays on NRP-152 and NRP-154 prostate cells

Task 3: To evaluate effects of new triterpenoids on the activity of the iNOS and COX-2 promoters
- Perform necessary CAT and luciferase assays with respective promoters

Task 4: Attempt to identify new receptors for triterpenoids

Synthesis of New Triterpenoids (Task 1)

We have made excellent progress in the synthesis of new synthetic triterpenoids during the past three years. We are attaching two reprints and a preprint to document this statement. The reprints are: "Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Gribble, G. W., Suh, N., Finlay, H. J., Rounds, B. V., Bore,

L., Favaloro, F. G., Wang, Y., and Sporn, M. B., published in *Journal of Medicinal Chemistry*, 43:1866-1877, 2000; and "Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Rounds, B. V., Bore, L., Finlay, H. J., Favaloro, F. G., Jr., Suh, N., Wang, Y., Sporn, M. B., and Gribble, G. W., published in *Journal of Medicinal Chemistry*, 43: 4233-4246, 2000; and the preprint is "A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrille, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production" by Honda, T., Honda, Y., Favaloro, F. G., Gribble, G. W., Suh, N., Place, A., Rendi, M., Sporn, M. B.; this preprint has just been submitted to *Bioorganic and Medicinal Chemistry Letters*. In aggregate, the three articles describe the synthesis and biological activity of more than 100 new synthetic triterpenoids, with special emphasis on suppression of the induction of iNOS (inducible nitric oxide synthase). The most recently made triterpenoids, such as compounds **4** and **28** in the preprint, are active in the picomolar (10^{-12} M) range. All three of the above articles acknowledge support from this grant, DAMD17-98-1-8604.

In addition to the pentacyclic triterpenoids described in the above reprints, we have also been attempting to simplify the structural requirements for inhibition of de novo synthesis for iNOS. Accordingly, we have just completed the synthesis of 9 new tricyclic structures, which are triterpenoid-like bis-enones. The structures of these new molecules, labeled TBE-001 to TBE-009, are attached. Please note that all 9 of these new molecules are simplified structural analogues of the highly potent triterpenoid, CDDO, which is described in the above mentioned reprints. The activity of TBE-009, as an inhibitor of induction of iNOS, is only 1 log less than that of CDDO itself, and we are therefore optimistic that this series of new analogues will be found to be useful agents themselves.

Biological Assays of New Triterpenoids (Task 2)

- Northern blots to measure suppression of de novo synthesis of iNOS mRNA. Data on page 13 show that when NRP-152 or NPR-154 prostate cells are treated with the combination of LPS (30 ng/ml) and TPA (30 ng/ml) there is major induction of new mRNA for iNOS. Simultaneous treatment of these prostate cells for 10 hours with CDDO (1 micromolar) or the triterpenoid, TP-82 (3 micromolar) causes almost total inhibition of this induction of iNOS. Page 13 also shows that the parent substance for the synthesis of CDDO or TP-82, namely oleanolic acid, is inactive in this regard.

- Western blots to measure suppression of de novo synthesis of iNOS protein. Data on pages 14 - 18 show results with Western blots for iNOS protein, which confirm the Northern blot data shown in the previous section. Moreover, page 14 also shows that CDDO and TP-82 are essentially inactive in blocking de novo induction of COX-2 protein in NRP-152 cells.

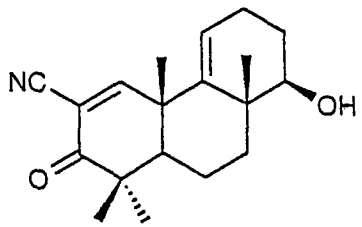
- Biological activity of new tricyclic bis-enone compounds (TBEs). Data on pages 19 and 20 show our first measurements of the biological activity of the new TBEs described above (TBE-1A, TBE-2A, TBE-3A, TBE-4A, and TBE-5A). The first measurements that we performed were to measure inhibition of nitric oxide production in primary macrophages that had been treated with interferon-γ (page 19). Peritoneal macrophages were treated with interferon–γ (40 ng/ml) to induce nitric oxide production simultaneously these macrophages were incubated with either CDDO or one of the 5 TBEs for 48 hours and then nitric oxide in the supernatant was measured by the Griess Reaction. Page 19 shows that TBE-5A has substantial inhibitory activity (greater than 50% at 100 nanomolar), while the other TBEs are somewhat less potent, although all show highly significant activity at 1 micromolar. TBEs have also been found, for the first time, to have substantial anti-proliferative activity on prostate cells. Thus, on page 20 we show that TBEs 3A, 4A, and 5A all cause almost total inhibition of thymidine incorporation into DNA in NRP-152 cells at a concentration of 1 micromolar.

Evaluation of Effects of New Triterpenoids on the Activity of the iNOS and COX-2 Gene Promoters (Task 3)

As proposed in Task 3, we have obtained data that the synthetic triterpenoid CDDO suppresses the activity of the iNOS and COX-2 gene promoters. The figure on page 21 shows data for effects of CDDO on the iNOS promoter linked to the luciferase, while the figure on page 22 shows similar data for the COX-2 promoter. The iNOS promoter is especially sensitive to CDDO.

Identification of New Receptors for Triterpenoids (Task 4)

We have also been able to show that the synthetic triterpenoid CDDO is a ligand for the nuclear receptor, PPAR-γ. These experiments have been done in collaboration with Dr. Timothy Willson and Dr. Steven Blanchard, Glaxo Wellcome, Research Triangle Park, NC. The figure on page 23 shows the result of a receptor-binding assay, which uses scintillation proximity technology for the measurement of ligands interacting with their receptors. Full results of these studies have been published in "A synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), is a ligand for the peroxisome proliferator-activated receptor γ." Wang, Y., Porter, W. W., Suh, N., Honda, T., Gribble, G. W., Leesnitzer, L. M., Plunket, K. D., Mangelsdorf, D. J., Blanchard, S. G., Willson, T. W., and Sporn, M. B., published in *Molecular Endocrinology*, 14: 1550-1556, 2000. At present, there is a great deal of interest in the potential role of PPAR-γ in prostate cancer, and in the future, we intend to explore our important finding of CDDO as a ligand for PPAR-γ. It is possible that PPAR-γ agonists and antagonists may turn out to be useful drugs for prevention or treatment of prostate cancer at some future date.

TBE-Number		TBE-001A	
Structure		Date	9 /3/ 99
		Quantity	10.0 mg
		Known ☐ Unknown ☑	
		Solubility	
		H_2O / EtOH / DMSO	
		>10 mg/mL ☐ ☐ ☐	
		1 mg/mL ☐ ☑ ☑	
		0.1 mg/mL ☐ ☐ ☐	
		<0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{19}H_{25}NO_2$		
Molecular Weight	299.41	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	> 95 % (by NMR)	Note No. Page	FF-I-208-1
IUPAC Name	(±)-3-Cyano-8β-hydroxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthren-2-one		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number		TBE-002A	
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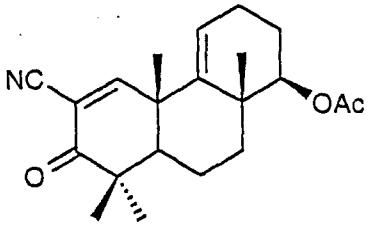
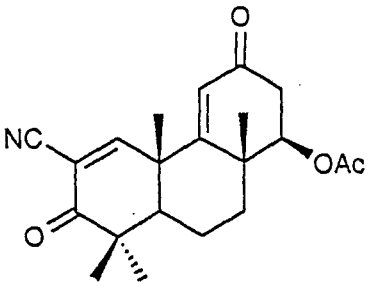
		Quantity	10.0 mg
		Known ☐ Unknown ☑	
		Solubility	
		H_2O / EtOH / DMSO	
		>10 mg/mL ☐ ☐ ☐	
		1 mg/mL ☐ ☑ ☑	
		0.1 mg/mL ☐ ☐ ☐	
		<0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{21}H_{27}NO_3$		
Molecular Weight	341.45	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	> 95 % (by NMR)	Note No. Page	FF-I-191-2
IUPAC Name	(±)-3-Cyano-8β-acetoxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthren-2-one		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number			TBE-003A
Structure		Date	9/9/99
		Quantity	7.6 mg
		Known ☐	Unknown ☑
		Solubility	
			H_2O EtOH DMSO >10 mg/mL ☐ ☐ ☐ 1 mg/mL ☐ ☑ ☑ 0.1 mg/mL ☐ ☐ ☐ <0.01 mg/mL ☑ ☐ ☐
Formula	$C_{21}H_{25}NO_4$		
Molecular Weight	355.43	Spectra	
mp		NMR ☑	MS ☐
$[\alpha]_D$		IR ☐	EA ☐
Form	Crystal ☐	Amorphous ☑	Oil ☐
Purity	> 95 % (by NMR)	Note No. Page	FF-I-206-2
IUPAC Name	(±)-3-Cyano-8β-acetoxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthrene-2,6-dione		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number		TBE-004A	
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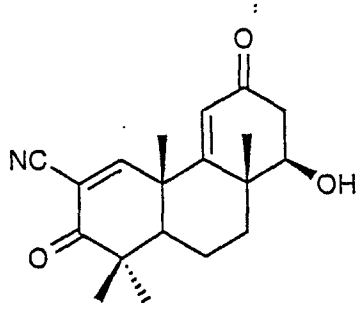		Quantity	7.6 mg
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		Solubility	
		H_2O / EtOH / DMSO	
		>10 mg/mL ☐ ☐ ☐	
		1 mg/mL ☐ ☑ ☑	
		0.1 mg/mL ☐ ☐ ☐	
		<0.01 mg/mL ☑ ☐ ☐	
Formula	$C_{19}H_{23}NO_3$		
Molecular Weight	313.40	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	95 % (by NMR)	Note No. Page	FF-I-207L
IUPAC Name	(±)-3-Cyano-8β-hydroxy-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,7,8,8a,9,10,10aα-decahydrophenanthrene-2,6-dione		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Favaloro, Jr.	Group Name	GWG

TBE-Number		TBE-005A	
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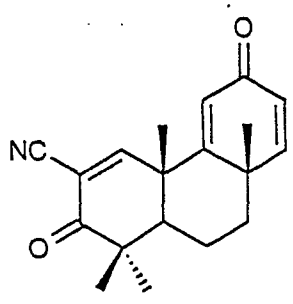		Quantity	7.5 mg
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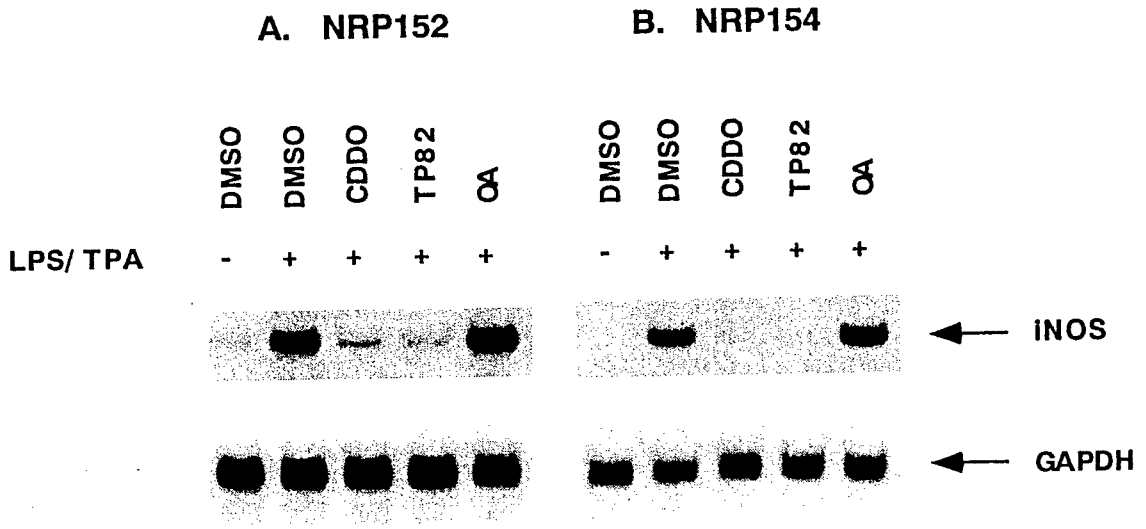
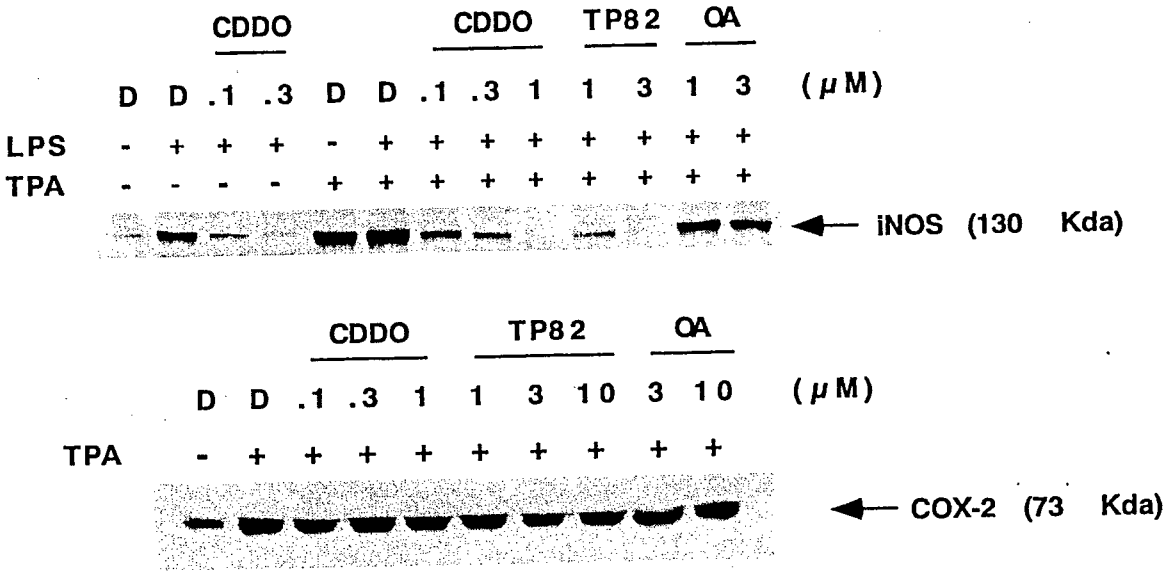
		1 mg/mL: ☐ ☑ ☑	
		0.1 mg/mL: ☐ ☐ ☐	
		<0.01 mg/mL: ☑ ☐ ☐	
Formula	$C_{19}H_{21}NO_2$		
Molecular Weight	295.38	Spectra	
mp		NMR ☑ MS ☐	
$[\alpha]_D$		IR ☐ EA ☐	
Form	Crystal ☐ Amorphous ☑ Oil ☐		
Purity	95 % (by NMR)	Note No. Page	FF-I-207H
IUPAC Name	(±)-3-Cyano-1,1,4aβ,8aβ-tetramethyl-1,2,4a,6,8a,9,10,10aα-octahydrophenanthrene-2,6-dione		
Remarks	Unstable against air. Store in freezer. Keep it under argon.		
Supplier	Frank G. Fayaloro, Jr.	Group Name	GWG

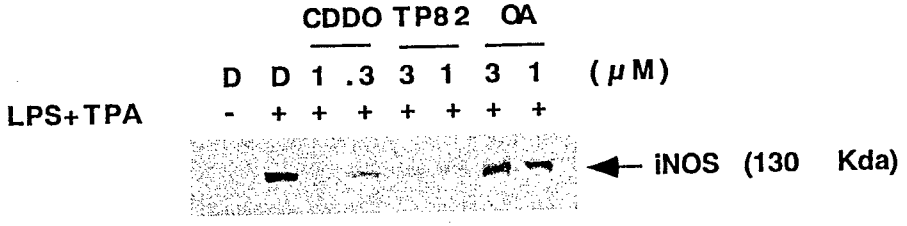


NRP152 cells were treated with LPS (30 ng/ml) and TPA (30 ng/ml) in the presence or absence of different concentrations of compounds (CDDO, 1 μM; TP-82, 3 μM; oleanolic acid, OA, 3 μM) for 10 h. mRNA were obtained and used for Northern analysis for iNOS expression.

A. NRP152

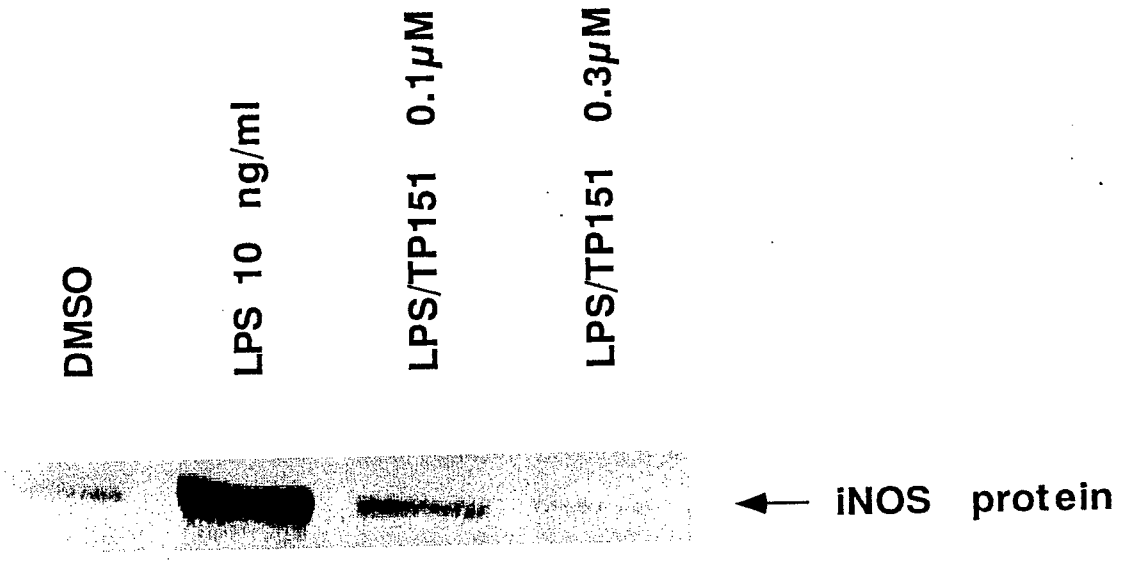


B. NRP154



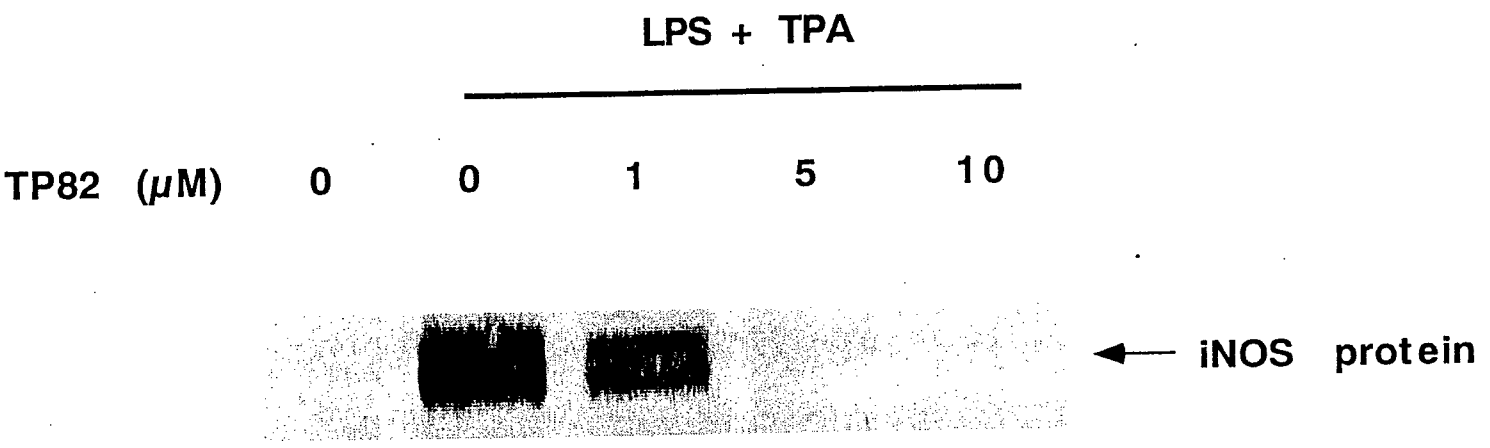
NRP152 and NRP-154 cells were treated with LPS (10 ng/ml), TPA (10 ng/ml) or with LPS plus TPA in the presence or absence of different concentrations of compounds for 12 h. Cell lysates were obtained and used for western analysis for iNOS or COX-2 expression.

Repression of iNOS protein by TP151 (CDDO) in NRP-152



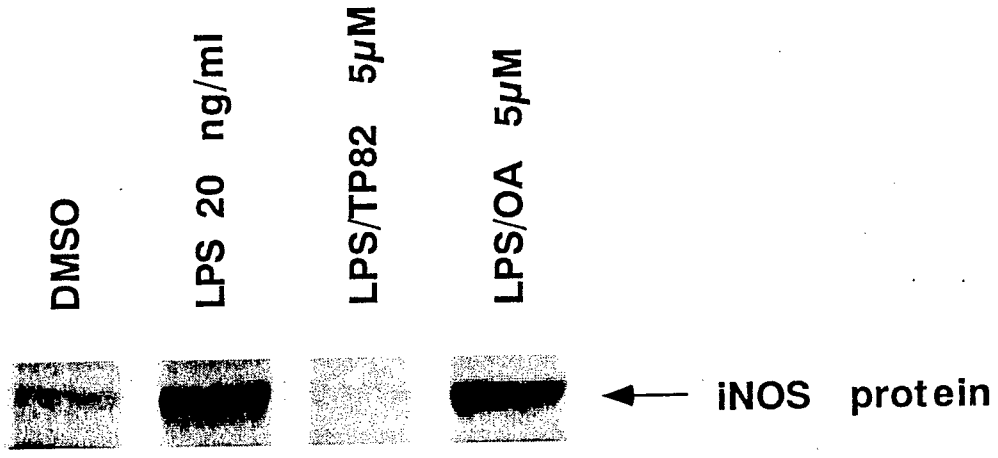
Exponentially growing NRP152 cells were treated with LPS with or without TP151 at indicated concentrations for 12 h. Cell lysates were harvested and subjected to western analysis.

Repression of iNOS by TP82 in NRP152



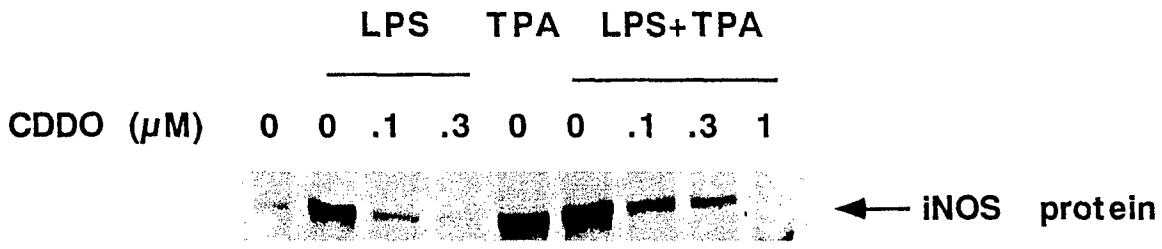
NRP152 cells were treated with LPS (10 ng/ml) and TPA (10 ng/ml) in the presence or absence of different concentrations of compounds for 12 h. Cell lysates were obtained and used for western analysis for iNOS or COX-2 expression.

Comparison of TP82 and OA in regulation of iNOS protein in NRP-152



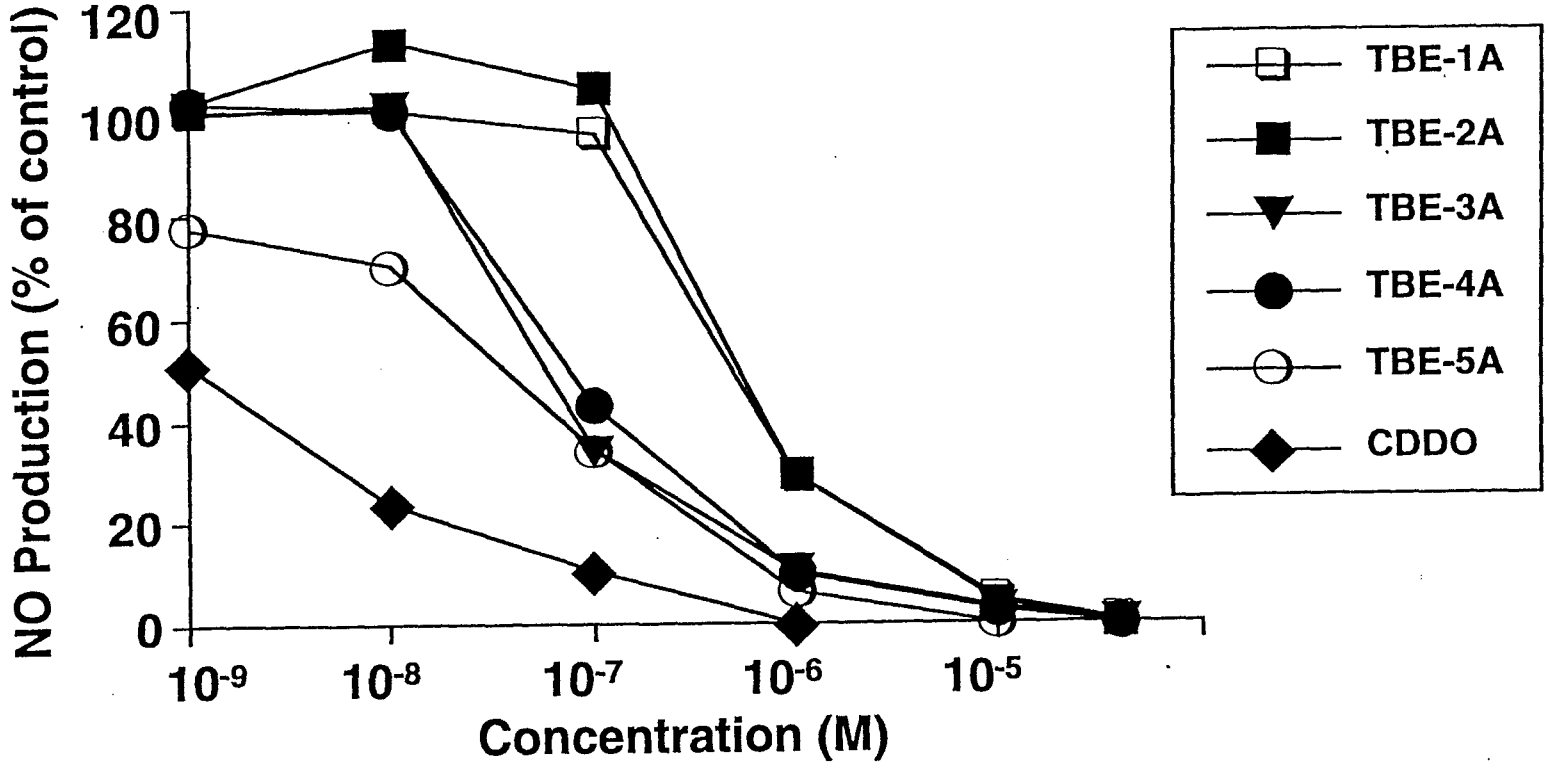
Exponentially growing NRP152 cells were treated with LPS, with LPS and TP82 combination or with LPS and OA combination at indicated concentrations for 12 h. Cell lysates were harvested and subjected to Western analysis.

Repression of iNOS protein by TP151 (CDDO) in NRP-152



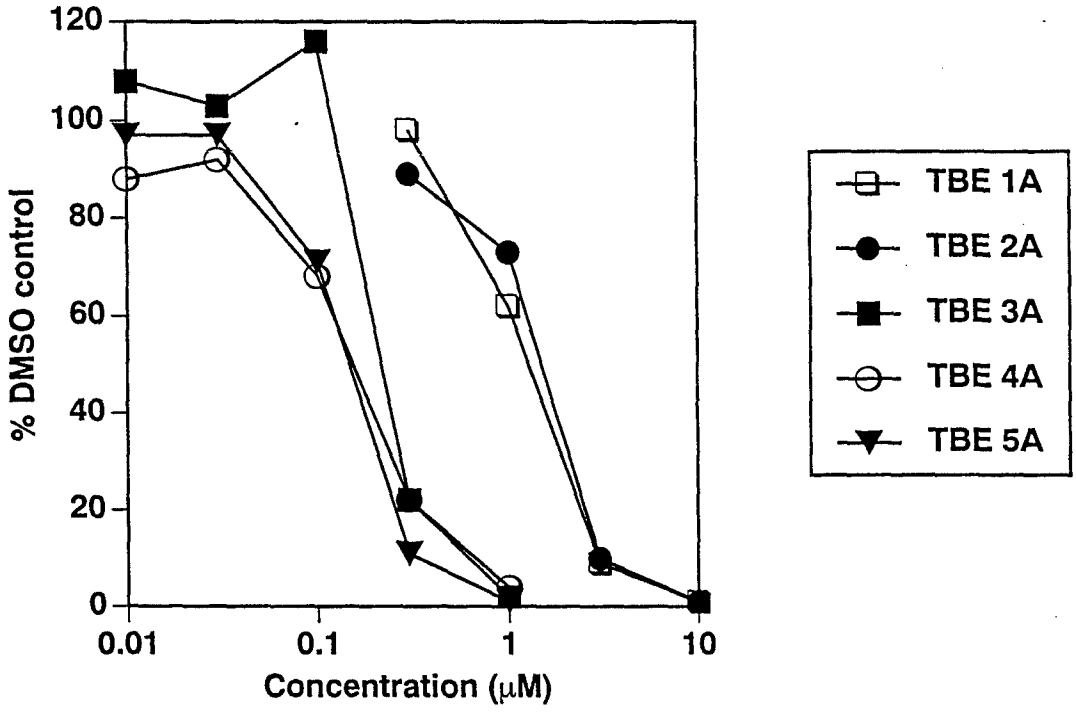
NRP152 cells were treated with LPS (10 ng/ml), TPA (10 ng/ml) or with LPS plus TPA in the presence or absence of different concentrations of TP151 (CDDO) for 12 h. Cell lysates were obtained and used for western analysis for iNOS expression.

TBEs inhibit nitric oxide production in primary mouse macrophages induced by interferon-γ



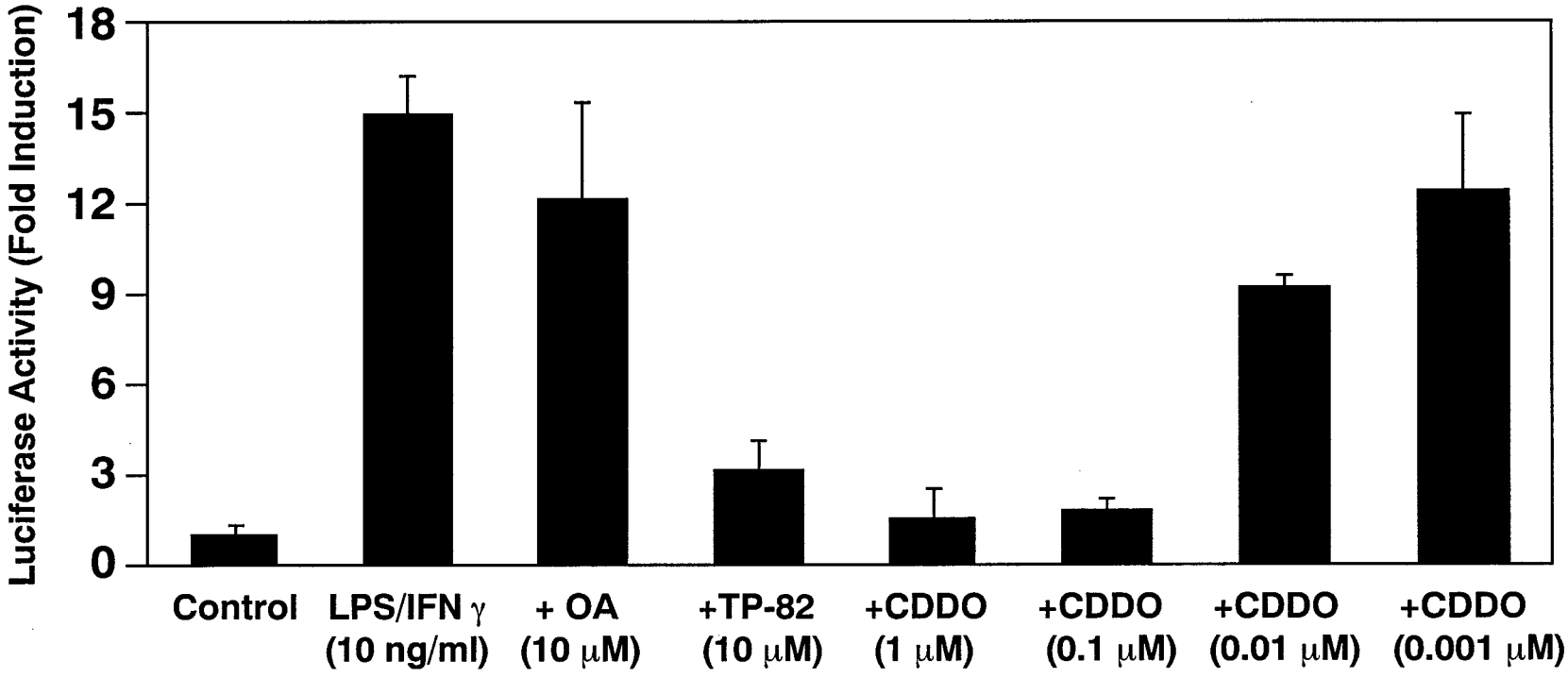
10/1/99 TBEs inhibit nitric oxide (NO) production in primary macrophages. IFN-γ (40 ng/ml) was used to induce nitric oxide production in mouse macrophages. The cells were incubated for 48 hrs with the inducer and compounds, then nitric oxide in the supernatant was measured by Griess Reaction.

Tricyclic Bis-Enone Compounds on NRP-152 Cells



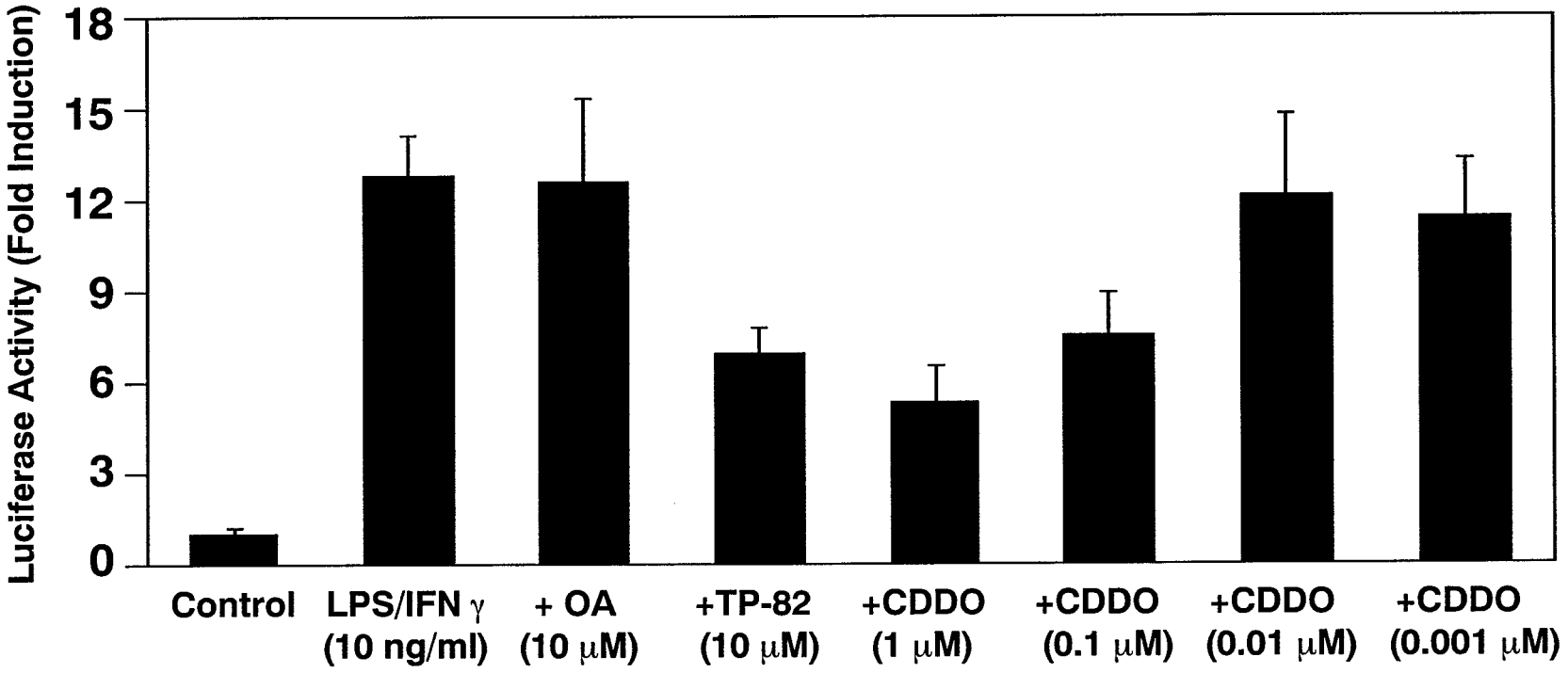
11-8-99 Tricyclic bis-enone (TBE) compounds inhibit growth of NRP-152 rat prostate cells. Cells were incubated with TBEs for 72 hours in DMEM/F12 media containing 1% charcoal-stripped serum, insulin, dexamethasone, and HEPES. Growth inhibition was measured by 3H-thymidine incorporation.

Triterpenoids Suppress iNOS (639 bp Fragment) Promoter Activities in RAW 264.7 Cells

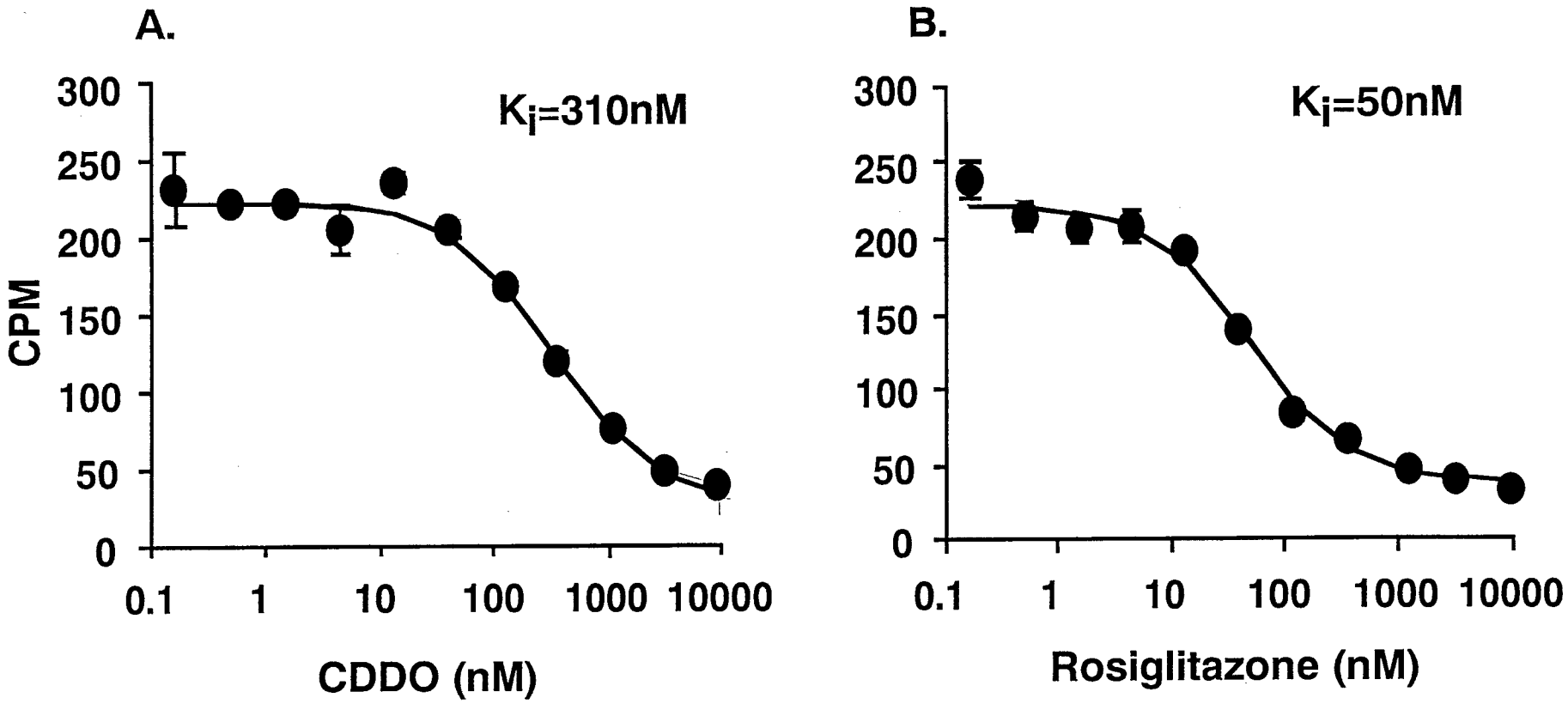


iNOS (639 bp fragment) promoter-Luc (50 ng DNA) and pCMX β-gal (50 ng DNA) were transfected into RAW 264.7 cells in 48-well plates. One day later, LPS and IFN-γ (10 ng/ml each) were added for 20 hrs together with triterpenoids. Luciferase acitivity was normalized by β-gal.

Triterpenoids Suppress TIS10L (COX-2) Promoter Activities in RAW 264.7 Cells



TIS10 L (COX-2) promoter-Luc (50 ng DNA) and pCMX β-gal (50 ng DNA) were transfected into RAW 264.7 cells in 48-well plates. One day later, LPS and IFN-γ (10 ng/ml each) were added for 20 hrs together with triterpenoids. Luciferase acitivity was normalized by β-gal.



CDDO binds to PPARγ. Non-radioactive CDDO (A) or rosiglitazone (B) was used to compete for binding to PPARγ using 50 nM [^{3}H] CDDO as the ligand. (in collaboration with Drs. Timothy Willson and Steven Blanchard, Glaxo Wellcome)

Key Research Accomplishments

- First synthesis of new tricyclic bis-enones structurally related to pentacyclic triterpenoids
- Demonstration of potent activity of new triterpenoid, CDDO, for suppression of growth of prostate epithelial cells
- Demonstration of potent activity of new triterpenoid, CDDO, for suppression of de novo induction of iNOS mRNA and iNOS protein in prostate epithelial cells
- Demonstration of ability of new tricyclic bis-enones to block induction of iNOS in primary mouse macrophages
- Demonstration of ability of new tricyclic bis-enones to inhibit growth of prostate epithelial cells
- Demonstration that CDDO suppresses the activity of the iNOS and COX-2 gene promoters
- Demonstration that CDDO is a ligand for the nuclear receptor PPAR-γ.

Reportable Outcomes

Published Manuscripts:

- "Novel synthetic oleanane triterpenoids, a series of highly active inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Rounds, B. V., Bore, L., Favaloro, F. G. Jr., Gribble, G. W., Suh, N., Wang, Y., and Sporn, M. B. Bioorganic & Medicinal Chemistry Letters. 9: 3429-3434, 1999.
- "Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Gribble, G. W., Suh, N., Finlay, H. J., Rounds, B. V., Bore, L., Favaloro, F. G., Wang, Y., and Sporn, M. B. Journal of Medicinal Chemistry, 43:1866-1877, 2000.
- "Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Rounds, B. V., Bore, L., Finlay, H. J., Favaloro, F. G., Jr., Suh, N., Wang, Y., Sporn, M. B., and Gribble, G. W. Journal of Medicinal Chemistry, 43: 4233-4246, 2000.
- "A synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), is a ligand for the peroxisome proliferator-activated receptor γ." Wang, Y., Porter, W. W., Suh, N., Honda, T., Gribble, G. W., Leesnitzer, L. M., Plunket, K. D., Mangelsdorf, D. J., Blanchard, S. G., Willson, T. W., and Sporn, M. B. , Molecular Endocrinology, 14: 1550-1556, 2000.

Manuscript Submitted:

- "A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrille, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production" by Honda, T., Honda, Y., Favaloro, F. G., Gribble, G. W., Suh, N., Place, A., Rendi, M., Sporn, M. B. Submitted to Bioorganic & Medicinal Chemistry Letters.

Conclusions

Synthetic triterpenoids represent an important class of new drugs that have potential for clinical use for prevention and treatment of prostate cancer. However, a great deal more research will need to be done before this will be clinically practical. In particular, a whole new set of pharmacokinetic studies will need to be done in the future.

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- Honda, T., Gribble, G. W., Suh, N., Finlay, H. J., Rounds, B. V., Bore, L., Favaloro, F. G., Wang, Y., and Sporn, M. B.: Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages. J. Med. Chem., 43:1866-1877, 2000.
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- Honda, T., Honda, Y., Favaloro, F. G., Gribble, G. W., Suh, N., Place, A., Rendi, M., Sporn, M. B. A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrille, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production. Unpublished manuscript submitted to Bioorganic & Medicinal Chemistry Letters.

List of Personnel Receiving Pay from this Grant

- Michael B. Sporn, M.D.
- Tadashi Honda, Ph.D.
- Nanjoo Suh, Ph.D.
- Renee Risingsong, B.S.
- Charlotte Williams, B.A.
- BarbieAnn Rounds, Ph.D.

Appendices

Appendix 1: "Novel synthetic oleanane triterpenoids, a series of highly active inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Rounds, B. V., Bore, L., Favaloro, F. G. Jr., Gribble, G. W., Suh, N., Wang, Y., and Sporn, M. B. Bioorganic & Medicinal Chemistry Letters. 9: 3429-3434, 1999.

Appendix 2: "Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Gribble, G. W., Suh, N., Finlay, H. J., Rounds, B. V., Bore, L., Favaloro, F. G., Wang, Y., and Sporn, M. B. Journal of Medicinal Chemistry, 43:1866-1877, 2000.

Appendix 3: "Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages" by Honda, T., Rounds, B. V., Bore, L., Finlay, H. J., Favaloro, F. G., Jr., Suh, N., Wang, Y., Sporn, M. B., and Gribble, G. W. Journal of Medicinal Chemistry, 43: 4233-4246, 2000.

Appendix 4: "A synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), is a ligand for the peroxisome proliferator-activated receptor γ." Wang, Y., Porter, W. W., Suh, N., Honda, T., Gribble, G. W., Leesnitzer, L. M., Plunket, K. D., Mangelsdorf, D. J., Blanchard, S. G., Willson, T. W., and Sporn, M. B. , Mol. Endocrinol., 14: 1550-1556, 2000.

Appendix 5: "A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrille, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production" by Honda, T., Honda, Y., Favaloro, F. G., Gribble, G. W., Suh, N., Place, A., Rendi, M., Sporn, M. B. Submitted to Bioorganic & Medicinal Chemistry Letters.

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL SYNTHETIC OLEANANE TRITERPENOIDS: A SERIES OF HIGHLY ACTIVE INHIBITORS OF NITRIC OXIDE PRODUCTION IN MOUSE MACROPHAGES

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Abstract: Novel oleanane triterpenoids with modified rings A and C were designed and synthesized. Among them, methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate showed similar high inhibitory activity (IC_{50} = 0.8 nM) to 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), which we have synthesized previously, against production of nitric oxide induced by interferon-γ in mouse macrophages.

Introduction

In a previous communication[1] we reported that 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) (**1**) has high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50} = 0.1 nM level). We also showed that CDDO is a potent, multifunctional agent.[2] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. CDDO inhibits proliferation of many human tumor cell lines. CDDO blocks *de novo* synthesis of inducible nitric oxide synthase (*i*-NOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. CDDO will protect rat brain hippocampal neurons from cell death induced by β-amyloid. The above activities have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture.

In the communication,[1] we also reported that the combination of a 1-en-3-one functionality with a nitrile group at C-2 in ring A and a 9-en-12-one functionality in ring C enhances activity very strongly in comparison with the enhancement by each functionality alone. We therefore designed and synthesized a series of novel oleanane triterpenoids to survey what combination of ring A with ring C provides highly active compounds. We have found that methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate (**2**) has similar high inhibitory activity to CDDO and methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO methyl ester) (**3**).[1,3] The new compound **2** is expected to be an alternative agent to CDDO. In this communication, the synthesis, inhibitory activity, and structure–activity relationships (SAR) are reported for these analogs.

Chemistry

Modification of Ring A (Schemes 1 and 2)

Initially, we designed and synthesized new olean-12-ene derivatives with a 1-en-3-one functionality having a substituent at C-2 in ring A, **6–9** and **12–18**, to discover which substituents enhance activity in comparison with the lead compound **4**, which was reported previously.[4] Chloride **6** was synthesized in 81% yield from

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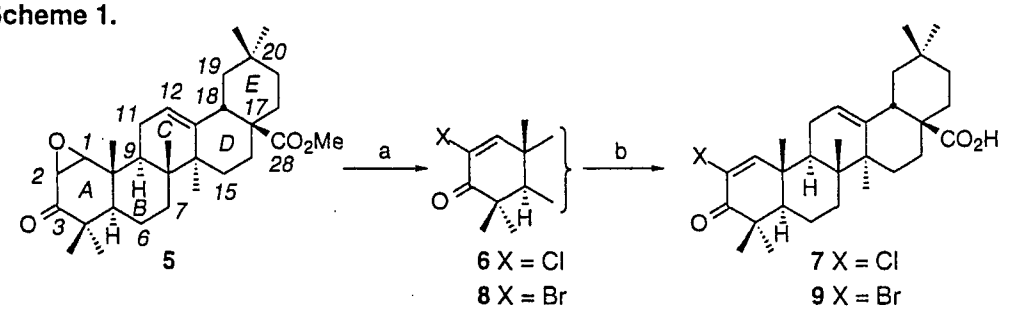
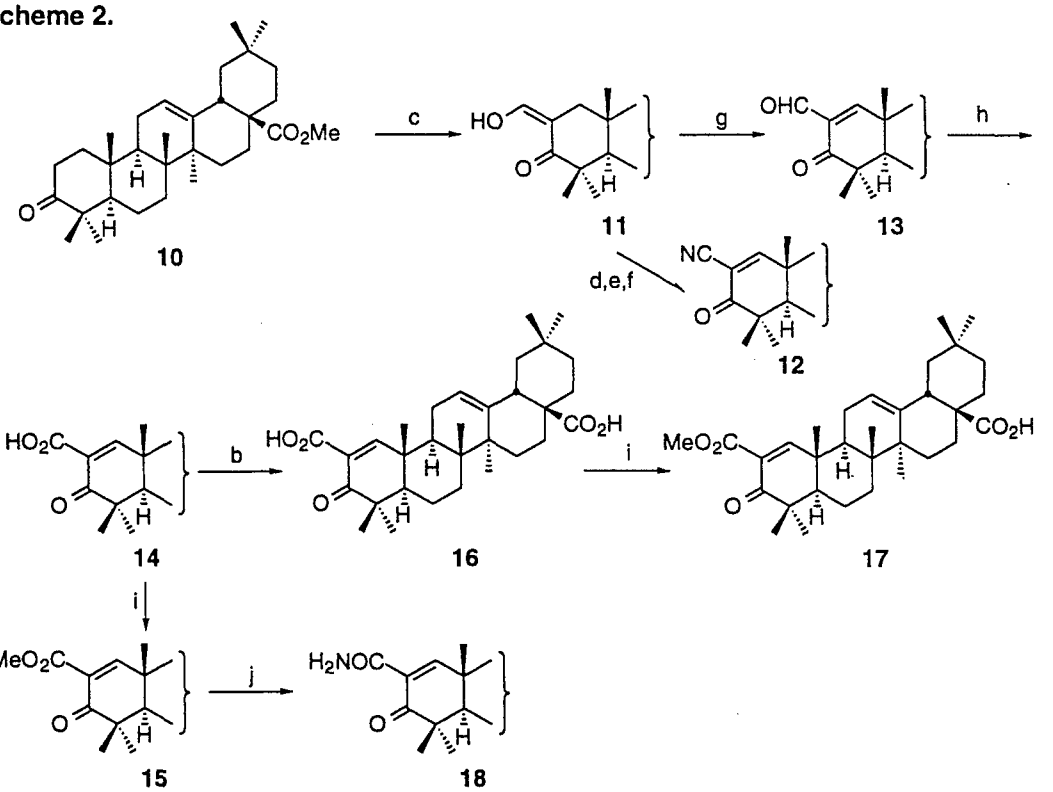
Scheme 1.

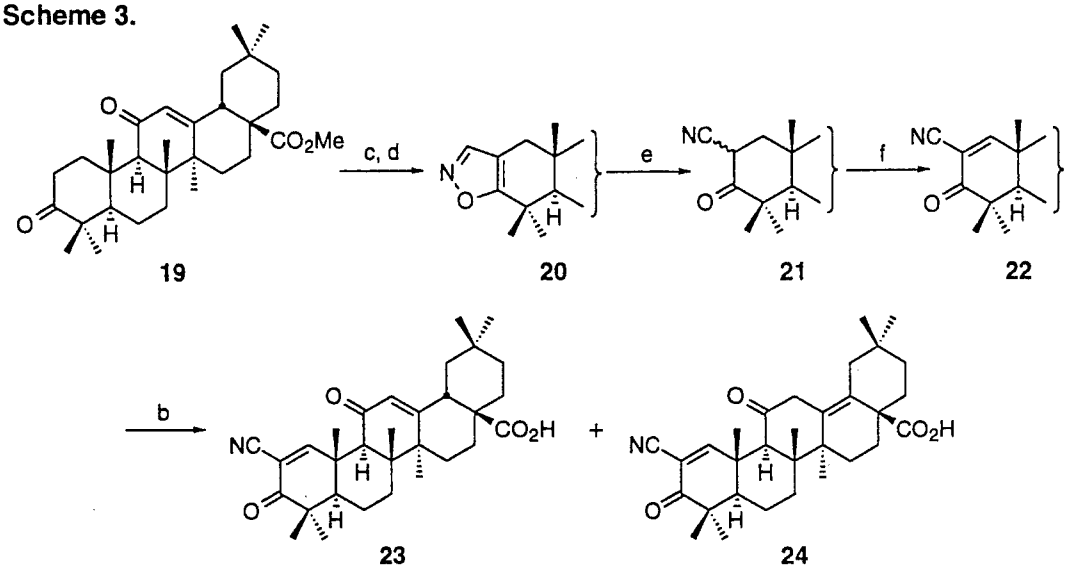


Scheme 2.

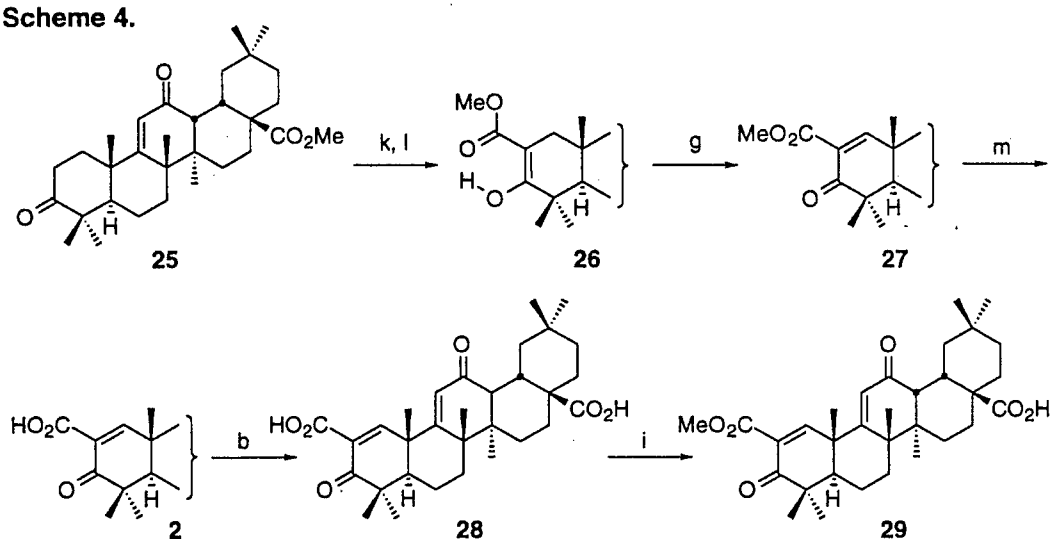


epoxide **5**[4] with hydrogen chloride in acetic acid and $CHCl_3$.[5] Halogenolysis of **6** with LiI in DMF[6] gave chloride **7** in 77% yield. Similarly, bromides **8** and **9** were prepared from **5** and **8** (yield, 96% and 76%), respectively. Compound **11**[7] was prepared in 95% yield by formylation of C-3 ketone **10**[4] with ethyl formate in the presence of sodium methoxide in benzene.[8] Nitrile **12** was synthesized in three steps (yield, 30%) from **11** according to the same synthetic route as for **30**, which was prepared previously.[1] Enal **13** was prepared from **11** by phenylselenenyl chloride-pyridine in CH_2Cl_2 and sequential addition of 30% H_2O_2[9] (yield, 71%; 79% based on recovered **11**). Jones oxidation of **13** gave acid **14** in 30% yield. Methylation of **14** with MeOH under acidic conditions gave ester **15** in 80% yield. Halogenolysis of **14** gave dicarboxylic acid **16** in 58% yield. Methylation of **16** with MeOH under acidic conditions gave ester **17** selectively in 70% yield because the carboxylic acid at C-17 of **16** is very sterically hindered. Amide **18** was prepared selectively in 72% yield from **15** with saturated ammonia-MeOH. Compounds **12** and **14–17** were found to be more active than the lead compound **4** (see Table 1).

Scheme 3.



Scheme 4.



a: HX/AcOH/$CHCl_3$, b: LiI/DMF, c: HCO_2Et/NaOMe/PhH, d: NH_2OH·HCl/aq EtOH, e: NaOMe/Et_2O/MeOH, f: PhSeCl/AcOEt; 30%H_2O_2/THF, g: PhSeCl/pyr./CH_2Cl_2; 30%H_2O_2/CH_2Cl_2, h: Jones, i: H_2SO_4/MeOH, j: NH_3/MeOH, k: Stiles' reagent/DMF, l: CH_2N_2/Et_2O/THF, m: KOH/aq MeOH

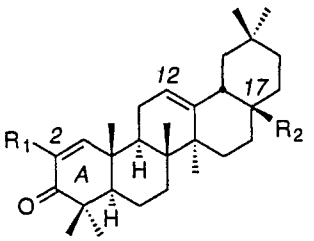
Modification of Ring C

We already reported the synthesis and inhibitory activity of 3-oxoolean-1-ene derivatives with various structures of ring C, and among them enones **31–33** are more active than the lead compound **4** (see Table 2).[4]

Combination of Modified Ring A with Ring C (Schemes 3 and 4)

On the basis of the above results, new oleanane derivatives with modified rings A and C, **2**, **22–24**, and **27–29**, were designed and synthesized. Isoxazole **20** was prepared from C-3 ketone **19**[4] by formylation (yield, 98%), followed by condensation with hydroxylamine (yield, 74%).[10] Cleavage of the isoxazole moiety of **20** with sodium methoxide gave nitrile **21** in 92% yield.[10] Nitrile **22** was prepared from **21** by phenylselenenyl

Table 1. IC_{50} (μM)[a] Values of Olean-12-ene Derivatives with Modified Ring A



compd	R_1 at C-2	R_2 at C-17	Taft's σ* value of R_1	activity IC_{50} (μM)
34[4]	OH	CO_2H	1.34	27
18	$CONH_2$	CO_2Me	1.68	14
35[4]	OMe	CO_2H	1.81	30
15	CO_2Me	CO_2Me	2	0.9
17	CO_2Me	CO_2H		2.2
14	CO_2H	CO_2Me	2.08	0.8
16	CO_2H	CO_2H		0.07
13	CHO	CO_2Me	2.15	toxic[b]
36[1]	CHO	CO_2H		toxic[b]
8	Br	CO_2Me	2.84	> 40
9	Br	CO_2H		7.3
6	Cl	CO_2Me	2.96	> 40
7	Cl	CO_2H		> 40
12	CN	CO_2Me	3.3	0.7
30[1]	CN	CO_2H		0.6
4[4]	H	CO_2H	-	5.6
oleanolic acid			-	> 40
hydrocortisone			-	0.01

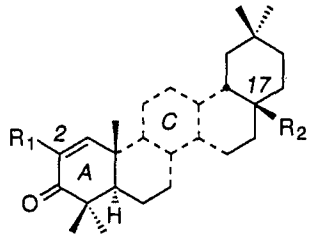
chloride in ethyl acetate and sequential addition of 30% H_2O_2[11] (yield, 33%; 57% based on recovered **21**). Halogenolysis of **22** gave acids **23** and **24** in 37% and 16% yield, respectively. Compounds **2** and **27–29** could not be synthesized according to the similar synthetic route as for **14–17** because Jones oxidation of the precursor of **2** (aldehyde at C-2) gives an unknown compound instead of **2**. They were synthesized according to the alternative route illustrated in Scheme 4. Ester **26** was prepared in 78% yield from C-3 ketone **25**[4] by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,[12] followed by methylation with diazomethane. Enone **27** was prepared from **26** according to the same method as for **13** (yield, 71%; 88% based on recovered **26**). Hydrolysis of **27** with potassium hydroxide in aqueous MeOH gave acid **2** selectively in 78% yield again because of the steric hindrance of the methoxycarbonyl group at C-17 of **27**. Halogenolysis of **2** gave dicarboxylic acid **28** and monocarboxylic acid **31** in 47% and 24% yield, respectively. Methylation of **28** with MeOH under acidic conditions gave ester **29** selectively in 82% yield.

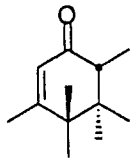
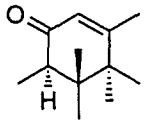
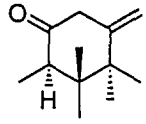
Biological Results and Discussion

Inhibitory Activity of Olean-12-ene Derivatives with Modified Ring A

The inhibitory activities [IC_{50} (μM) value] of olean-12-ene derivatives with a 1-en-3-one functionality with a substituent at C-2 in ring A,[13] oleanolic acid, and hydrocortisone (a positive control) on production of NO induced by IFN-γ in mouse macrophages[14] are shown in Table 1. These derivatives are arranged according to

Table 2. IC_{50} (μM)[a] Values of Oleanane Derivatives with Modified Rings A and C



compd	structure of ring C	R_1 at C-2	R_2 at C-17	activity IC_{50} (μM)
3 [1]		CN	CO_2Me	0.0001
1 [1]		CN	CO_2H	0.0002
27		CO_2Me	CO_2Me	toxic[b]
29		CO_2Me	CO_2H	0.1
2		CO_2H	CO_2Me	0.0008
28		CO_2H	CO_2H	0.2
31 [4]		H	CO_2H	0.2
22		CN	CO_2Me	0.02
23		CN	CO_2H	0.04
32 [4]		H	CO_2H	1.4
24		CN	CO_2H	0.07
33 [4]		H	CO_2H	2.6
dexamethasone				0.0001

[a]IC_{50} (μM) values of compounds **1–3**, **16**, **22–24**, hydrocortisone and dexamethasone were determined in the range of 0.1 pM–1 μM (tenfold dilutions). The other compounds were assayed in the range of 0.01–40 μM (fourfold dilutions). Values are an average of two separate experiments.
[b]Compounds **13**, **27** and **36** were toxic to cells above 1 μM and were not active below 1 μM.

the strength of Taft's σ* values[15] of substituents at C-2. These results provide the following interesting SAR:

(1) The relationship between Taft's σ* value and activity is not observed.

(2) Methoxycarbonyl, carboxyl, and nitrile groups at C-2 enhance activity. Compounds **12**, **14–16**, and **30** are about 10–100 times more active than the lead compound **4**.

(3) Hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decrease activity.

(4) Formyl group does not show activity, but only toxicity.

(5) Methoxycarbonyl and carboxyl groups at C-17 show similar activity.

Inhibitory Activity of Oleanane Derivatives with Modified Rings A and C

The inhibitory activities [IC_{50} (μM) value] of oleanane derivatives with modified rings A and C,[13] and dexamethasone (a positive control) on production of NO induced by IFN-γ in mouse macrophages are shown in Table 2. These results provide the following interesting SAR:

(1) A 9-en-12-one functionality is the strongest enhancer of activity among structures of ring C. Compound **31** is about 10 times more active than **4**.

(2) 12-En-11-one and 13-en-11-one functionalities also enhance activity. Compounds **32** and **33** are about 2–4 times more active than **4**.

(3) The combination of a 9-en-12-one functionality with nitrile and carboxyl groups at C-2 provides extremely highly active compounds. Compounds **2**, **3**, and CDDO (**1**) are about 10,000 times more active than **4**.

(4) The combination of 12-en-11-one and 13-en-11-one functionalities with a nitrile group at C-2 also provides highly active compounds. Compounds **22–24** are about 100 times more active than **4**.

(5) Although compounds **27–29** were also expected to show similar high activity to CDDO from the perspective of SAR, they did not show high activity.

Currently, further evaluation in vivo for both antiinflammation and chemoprevention of CDDO, **2**, and **3** are in progress. Studies on the mode of action of these compounds also are in progress.

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3. CDDO methyl ester (**3**) was found to show the same high activity as CDDO after our previous communication[1] was published.
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13. All new compounds, **2**, **6–9**, **12–18**, **22–24**, and **27–29** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses.
14. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days earlier with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 20 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 16.
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Novel Synthetic Oleanane and Ursane Triterpenoids with Various Enone Functionalities in Ring A as Inhibitors of Nitric Oxide Production in Mouse Macrophages

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Novel Synthetic Oleanane and Ursane Triterpenoids with Various Enone Functionalities in Ring A as Inhibitors of Nitric Oxide Production in Mouse Macrophages†

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We initially randomly synthesized about 60 oleanane and ursane triterpenoids as potential anti-inflammatory and cancer chemopreventive agents. Preliminary screening of these derivatives for inhibition of production of nitric oxide induced by interferon-γ in mouse macrophages revealed that 3-oxooleana-1,12-dien-28-oic acid (**B-15**) showed significant activity (IC_{50} = 5.6 μM). On the basis of the structure of **B-15**, 19 novel olean- and urs-12-ene triterpenoids with a 1-en-3-one functionality having a substituent at C-2 in ring A have been designed and synthesized. Among them, 3-oxooleana-1,12-diene derivatives with carboxyl, methoxycarbonyl, and nitrile groups at C-2 showed higher activity than the lead compound **B-15**. In particular, 2-carboxy-3-oxooleana-1,12-dien-28-oic acid (**3**) had the highest activity (IC_{50} = 0.07 μM) in this group of triterpenoids. The potency of **3** was similar to that of hydrocortisone (IC_{50} = 0.01 μM), although **3** does not act through the glucocorticoid receptor. Interesting structure–activity relationships of these novel synthetic triterpenoids are also discussed.

Introduction

Oleanane and ursane triterpenoids are pentacyclic compounds with 30 carbon atoms, which are derived biosynthetically by the cyclization of squalene.[1] The group includes a very large number of naturally occurring members that cover an impressive variety of functional groups.[2] Many compounds of this group are reported to have interesting biological, pharmacological, or medicinal activities similar to those of retinoids and steroids, such as anti-inflammatory activity, suppression of tumor promotion, suppression of immunoglobulin synthesis, protection of the liver against toxic injury, induction of collagen synthesis, and induction of differentiation in leukemia or teratocarcinoma cells.[3] However, the potency of these triterpenoids is relatively weak. There are no systematic studies of structure–activity relationships based on chemical modification of oleanane and ursane triterpenoids.[4] We have therefore considered that bioassay-directed systematic drug design and synthesis of derivatives of oleanolic acid (**1**) and ursolic acid (**2**), which are commercially available, could be of great value in discovering novel structures with high biological potency.

The high output of nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS), which is expressed in activated macrophages, plays an important role in host defense. However, excessive production of NO also can destroy functional normal tissues during acute and chronic inflammation.[5] This phenomenon is also closely related mechanistically to carcinogenesis.[6] Thus, inhibitors of NO production in macrophages are potential anti-inflammatory and cancer chemopreventive drugs. Because oleanolic and ursolic acids are already known to have weak anti-inflammatory and anticarcinogenic activity,[3a,3b,3e,3f] we focused our attention on therapeutic agents of these diseases. For this purpose, we have adopted an assay system that measures inhibition of NO production induced by interferon-γ (IFN-γ) in mouse macrophages[7] as a preliminary screening assay system. We synthesized various oleanolic and ursolic acid derivatives and tested them as inhibitors of NO production. As a result, we have identified a series of novel olean-12-ene triterpenoids with a 1-en-3-one functionality having carboxyl, methoxycarbonyl, and nitrile groups at C-2 in ring A that show significant inhibitory activity (IC_{50} = 0.01–0.1 μM level) against production of NO induced by IFN-γ in mouse macrophages. In particular, 2-carboxy-3-oxooleana-1,12-dien-28-oic acid (**3**) had the highest activity (IC_{50} = 0.07 μM) in this group of compounds. The potency of **3** was similar to that of hydrocortisone (IC_{50} = 0.01 μM), although **3** does not act through the glucocorticoid receptor. We report here the synthesis, inhibitory activity, and structure–activity relationships of these novel triterpenoids in detail.

Chemistry

Discovery of Lead Compound. When we started this project, we had no information about a lead

† Part of this work has been reported in preliminary form: (a) Honda, T.; Finlay, H. J.; Gribble, G. W.; Suh, N.; Sporn, M. B. New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1623–1628. (b) Honda, T.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. Novel synthetic oleanane triterpenoids: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3429–3434.

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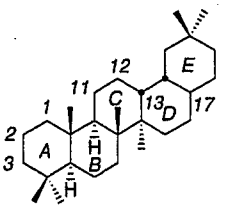
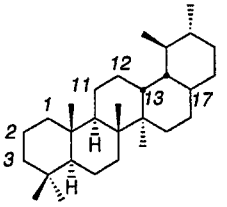
Table 1. Preliminary Screening Results of Synthetic Oleanane and Ursane Triterpenoids



oleanane



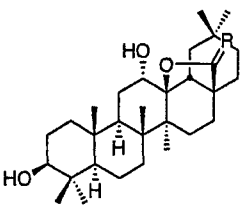
ursane

compd	skeleton	C-3	C-12	C-13	C-17	inhibition (%) at 10 μM[b]	ref
1	olean-12-ene	β-OH	H		CO_2H	38	10
2	urs-12-ene	β-OH	H		CO_2H	0	15
A-1	olean-12-ene	β-OH	H		CO_2Me	0	10
A-2	urs-12-ene	β-OH	H		CO_2Me	0	15
A-3	olean-12-ene	β-OAc	H		CO_2Me	10	10
A-4	urs-12-ene	β-OAc	H		CO_2Me	15	15
A-5	olean-12-ene	β-OAc	H		CO_2H	0	10
A-6	urs-12-ene	β-OAc	H		CO_2H	0	15
A-7	olean-12-ene	β-OH	H		CH_2OH	0	28
A-8	urs-12-ene	β-OH	H		CH_2OH	8	29
A-9	olean-12-ene	β-OAc	H		CH_2OAc	4	28
A-10	urs-12-ene	β-OAc	H		CH_2OAc	0	29
A-11	oleanane	β-OAc	α-OH	β-H	CO_2Me	0	*c*
A-12	oleanane	β-OAc	β-OH	β-H	CO_2Me	0	*c*
A-13	oleanane	β-OAc	β-OAc	β-H	CH_2OAc	0	*c*
A-14	oleanane	β-OAc	α-OH	β-H	CH_2OAc	0	*c*
A-15	oleanane	β-OH	α-OH	β-H	CH_2OH	48	*c*
A-16	oleanane	β-OH	β-OH	β-H	CH_2OH	20	*c*
A-17	oleanane	β-OH	=O	β-H	CO_2Me	0	10
A-18	oleanane	β-OAc	=O	β-H	CO_2Me	0	10
A-19	olean-12-ene	α-OH	H		CO_2H	18	30
A-20	urs-12-ene	α-OH	H		CO_2H	48	31
A-21[a]	oleanane	β-OH	α-OH	–O–	–CH(OH)–	21	32
A-22[a]	oleanane	β-OH	α-OH	–O–	–CO–	13	32
A-23	oleanane	β-OAc	12α,13α-epoxy-		CO_2Me	0	10
A-24	oleanane	β-OH	α-OH	β-OH	CH_2OH	22	32
B-1	olean-12-ene	=O	H		CO_2H	16	10
B-2	urs-12-ene	=O	H		CO_2H	22	33
B-3	olean-12-ene	=O	H		CO_2Me	24	10
B-4	urs-12-ene	=O	H		CO_2Me	16	15
B-5	olean-12-ene	=O	H		CHO	11	34
B-6	urs-12-ene	=O	H		CHO	21	34
B-7	oleana-11,13(18)-diene	=O	H		CO_2H	47	*c*
B-8	oleanane	=O	=O	β-H	CO_2Me	3	10
B-9	oleanane	=O	=O	β-H	CO_2H	37	*c*
B-10	oleanane	=O	=O	β-H	CHO	38	*c*
B-11[a]	oleanane	=O	α-Br	–O–	–CO–	4	10
B-12[a]	oleanane	=O	=O	–O–	–CO–	0	10
B-13	oleana-1,12-diene	=O	H		CO_2Me	19	9
B-14	ursa-1,12-diene	=O	H		CO_2Me	0	*d*
B-15	oleana-1,12-diene	=O	H		CO_2H	85	*d*
B-16	ursa-1,12-diene	=O	H		CO_2H	41	14
C-1[a]	urs-12-ene	=O	H		CO_2H	55	*c*
C-2	olean-12-ene	α-Cl	H		CO_2Me	2	*c*
C-3	olean-12-ene	α-Cl	H		CO_2H	0	*c*
D-1	oleana-2,12-diene	H	H		CO_2Me	3	35
D-2	oleana-2,12-diene	H	H		CO_2H	0	*c*
D-3[a]	olean-12-ene		H		CO_2H	0	*c*
E-1[a]	A-ring cleaved olean-12-ene		H		CO_2Me	21	36
E-2[a]	A-ring cleaved olean-12-ene		H		CO_2H	33	37
E-3[a]	A-ring cleaved urs-12-ene		H		CO_2H	39	37
E-4[a]	A-ring cleaved olean-12-ene		H		CO_2H	22	37
E-5[a]	A-ring cleaved urs-12-ene		H		CO_2H	55	37
E-6[a]	A-ring cleaved urs-12-ene		H		CO_2H	10	37
F-1[a]	C-ring cleaved oleanane	β-OAc			CH_2OAc	52	*c*
F-2[a]	C-ring cleaved oleanane	β-OAc			CH_2OAc	12	*c*
F-3[a]	C-ring cleaved oleanane	β-OAc			CH_2OAc	52	*c*
F-4[a]	C-ring cleaved oleanane	=O			CO_2H	28	*c*

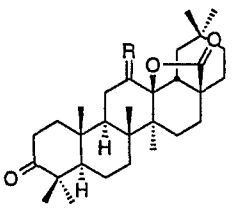
Table 1 (Continued)

compd	skeleton	C-3	C-12	C-13	C-17	inhibition (%) at 10 μM[b]	ref
G-1[a]	olean-12-ene		H		CO_2Me	0	36
G-2[a]	olean-12-ene		H		CO_2H	51	37
hydrocortisone						80	

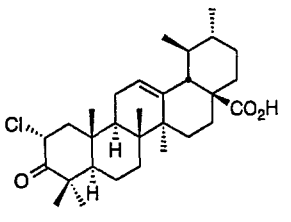
[a] Structure shown below this table. [b] Details of the evaluation method are described in the Experimental Section. [c] Unknown compound (synthesis and spectral data will be published elsewhere). [d] Unknown compound (synthesis and spectral data are shown in this paper).



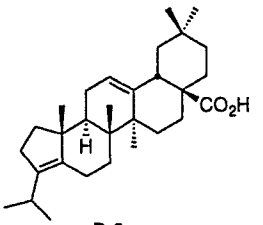
A-21: R = OH, H
A-22: R = O



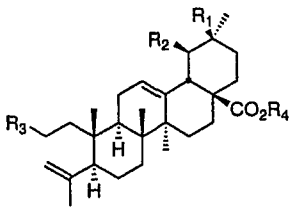
B-11: R = α-Br, β-H
B-12: R = O



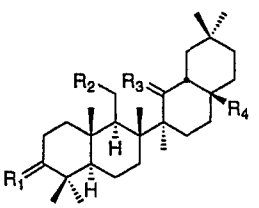
C-1



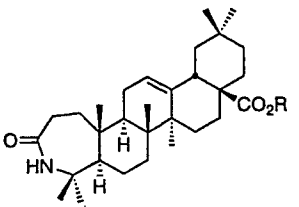
D-3



E-1: $R_1 = R_4$ = Me, R_2 = H, R_3 = CN
E-2: R_1 = Me, $R_2 = R_4$ = H, R_3 = CN
E-3: $R_1 = R_4$ = H, R_2 = Me, R_3 = CN
E-4: R_1 = Me, $R_2 = R_4$ = H, R_3 = CHO
E-5: $R_1 = R_4$ = H, R_2 = Me, R_3 = CHO
E-6: $R_1 = R_4$ = H, R_2 = Me, R_3 = CO_2H

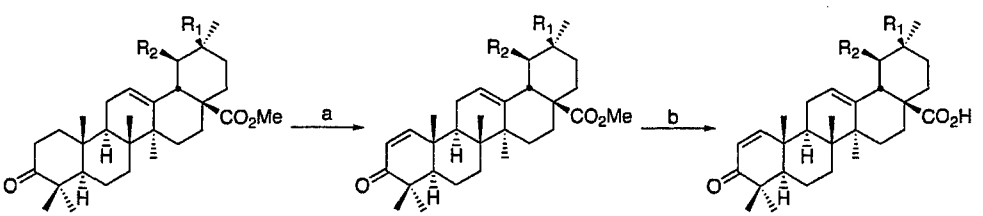


F-1: R_1 = β-OAc, R_2 = CHO, R_3 = O, R_4 = CH_2OAc
F-2: R_1 = β-OAc, R_2 = CH_2OH, R_3 = OH, R_4 = CH_2OAc
F-3: R_1 = β-OAc, R_2 = CO_2H, R_3 = O, R_4 = CH_2OAc
F-4: $R_1 = R_3$ = O, R_2 = CO_2H, R_4 = CO_2H



G-1: R = Me
G-2: R = H

Scheme 1[a]



B-3: R_1 = Me, R_2 = H
B-4: R_1 = H, R_2 = Me

B-13: R_1 = Me, R_2 = H
B-14: R_1 = H, R_2 = Me

B-15: R_1 = Me, R_2 = H
B-16: R_1 = H, R_2 = Me

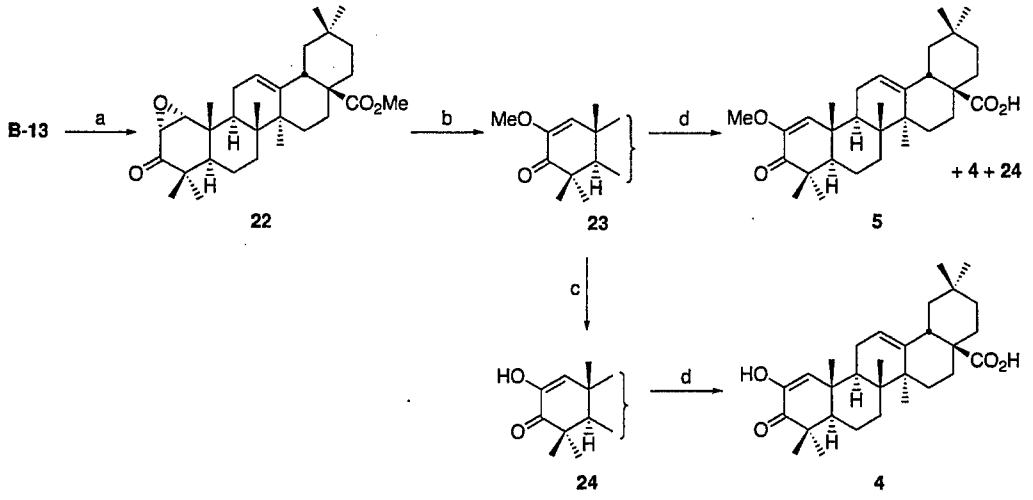
[a] Reagents: (a) PhSeCl, EtOAc; *m*CPBA, pyr, EtOAc; (b) LiI, DMF.

compound. Therefore, about 60 oleanolic and ursolic acid derivatives were initially randomly synthesized. They are divided into seven categories: 3-hydroxy derivatives, **A**; 3-oxo derivatives, **B**; chloro derivatives, **C**; dehydroxyoleanane derivatives, **D**; A-ring cleaved derivatives, **E**; C-ring cleaved oleanane derivatives, **F**; and lactams, **G** (see Table 1). In the preliminary screen of these derivatives for inhibition of production of NO induced by IFN-γ in mouse macrophages, 3-oxooleana-1,12-dien-28-oic acid (**B-15**) was found to show significant activity (inhibition: 85% at 10 μM, IC_{50} = 5.6 μM). (See Tables 1 and 2.)

Design and Synthesis of New Derivatives. When **B-15** is compared with the other derivatives, it has the following features: first, it is an oleanane; second, it has a 1-en-3-one functionality in ring A; third, it has a carboxyl group at C-17. We focused our attention on the 1-en-3-one functionality in ring A among these features. We therefore designed novel olean- and urs-12-ene triterpenoids with a 1-en-3-one functionality having a substituent at C-2 in ring A, **3**–**19**, and novel triterpenoid–steroid hybrid compounds, **20** and **21**[8] (see Table 2). The syntheses of these newly designed derivatives and compounds **B-13**–**B-16** are illustrated in Schemes 1–6.

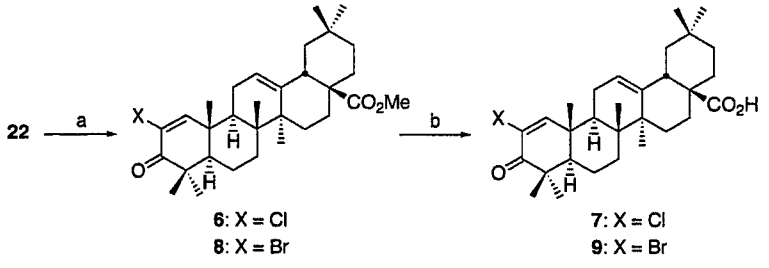
Ester **B-13**[9] was synthesized in 62% yield by introduction of a double bond at C-1 of methyl oleanonate (**B-3**)[10] with phenylselenenyl chloride (PhSeCl) in ethyl acetate and sequential addition of pyridine and *m*-chloroperbenzoic acid.[11,12] Acid **B-15** was synthesized in 85% yield by halogenolysis of **B-13** with lithium iodide in *N*,*N*-dimethylformamide (DMF).[13] Similarly, acid **B-16**[14] was synthesized in 58% yield via ester **B-14** from methyl ursonate (**B-4**).[15] Epoxide **22**[9] was prepared in 99% yield by epoxidation of **B-13** with alkaline hydrogen peroxide. Treatment of **22** with sodium methoxide[16] gave enone **23** (yield, 87%; 98% based on recovered **22**). Diosphenol **24** was synthesized by demethylation of the methyl enol ether at C-2 of **23** with hydrochloric acid in acetic acid (yield, 81%). Halogenolysis of **24** gave acid **4** (yield, 18%). Halogenolysis of **23** gave a desired partial demethylated product **5** in 28% (41% based on recovered

Scheme 2[a]



[a] Reagents: (a) 30% H_2O_2, NaOH(aq), THF; (b) NaOMe, MeOH; (c) HCl, AcOH; (d) LiI, DMF.

Scheme 3[a]



[a] Reagents: (a) HX, AcOH, $CHCl_3$; (b) LiI, DMF.

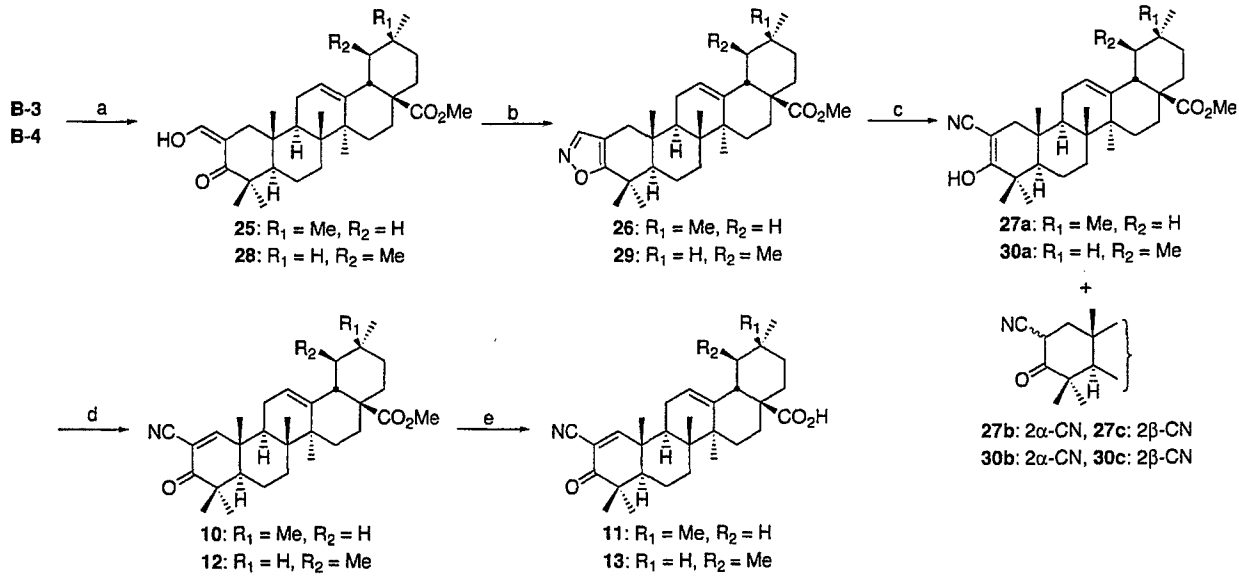
23) yield.[17] Chloride **6** was synthesized in 81% yield from **22** with hydrogen chloride in acetic acid and chloroform.[18] Halogenolysis of **6** gave chloride **7** in 77% yield. Similarly, bromides **8** and **9** were prepared from **22** and **8** (yield, 96% and 76%), respectively. Hydroxymethylene **25**[19,20] was prepared in 95% yield by formylation of **B-3** with ethyl formate in the presence of sodium methoxide in benzene.[21] Isoxazole **26** was prepared in 86% yield by condensation of **25** with hydroxylamine.[22] Cleavage of the isoxazole moiety of **26** with sodium methoxide gave nitrile **27** in 99% yield.[22] ^{1}H NMR showed that **27** is a mixture of three tautomers [**27a**, **27b** (2α-cyano), and **27c** (2β-cyano)] and that **27a** is the major one in $CDCl_3$. Enone **10** was prepared in 88% yield by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **27** in benzene, although the same method as for **B-13** gave **10** in only 35% yield. Halogenolysis of **10** gave acid **11** in 71% (91% based on recovered **10**) yield. Similarly, ursane derivative **12** was synthesized in 52% yield via **28**,[20,23] **29**, and **30** from **B-4**. Acid **13** was prepared in 74% yield by halogenolysis of **12**. Enal **14** was prepared from **25** by PhSeCl–pyridine in methylene chloride and sequential addition of 30% hydrogen peroxide[24] (yield, 71%; 79% based on recovered **25**). Halogenolysis of **14** did not give acid **15** but a complex mixture. Therefore, the synthesis of acid **15** from oleanonic acid (**B-1**)[10] was attempted. Formylation of **B-1** with ethyl formate in the presence of sodium methoxide in tetrahydrofuran gave **32**[20] (yield, 45%; 66% based on recovered **B-1**). Acid **15** was prepared from **32** according to the same method as for **14** (yield, 71%; 84% based on recovered **32**). Jones oxidation of **14** gave acid **16** in 30% (39% based on recovered **14**) yield. Because this yield was not enough to synthesize derivatives **3** and **17**–**19** from **16**, an alternative route was adopted. Ester **31** was prepared in 74% (89% based on recovered **B-3**) yield from **B-3** by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,[25] followed by methylation with diazomethane. ^{1}H NMR showed that **31** is the single tautomer in $CDCl_3$ as depicted in Scheme 5. Enone **17** was prepared from **31** according to the same method as for **14** (yield, 83%; 90% based on recovered **31**). Hydrolysis of **17** with potassium hydroxide in aqueous methanol gave acid **16** selectively in 97% yield because the methoxycarbonyl group at C-17 of **17** is sterically hindered. Halogenolysis of **16** gave dicarboxylic acid **3** in 58% yield. Methylation of **3** with methanol under acidic conditions gave ester **18** selectively in 78% yield because of the steric hindrance of the carboxylic acid at C-17 of **3**. Amide **19** was prepared selectively in 96% yield from **17** with saturated ammonia–methanol.

Biological Results and Discussion

The inhibitory activities [IC_{50} (μM) value] of compounds **B-1**, **B-13**, **B-15**, **B-16**, **1**–**21**, and hydrocortisone (a positive control) on NO production induced by IFN-γ in mouse macrophages are shown in Table 2. These derivatives are arranged according to the strength of Taft's σ* values[26] of substituents at C-2. These results provide the following interesting structure–activity relationships:

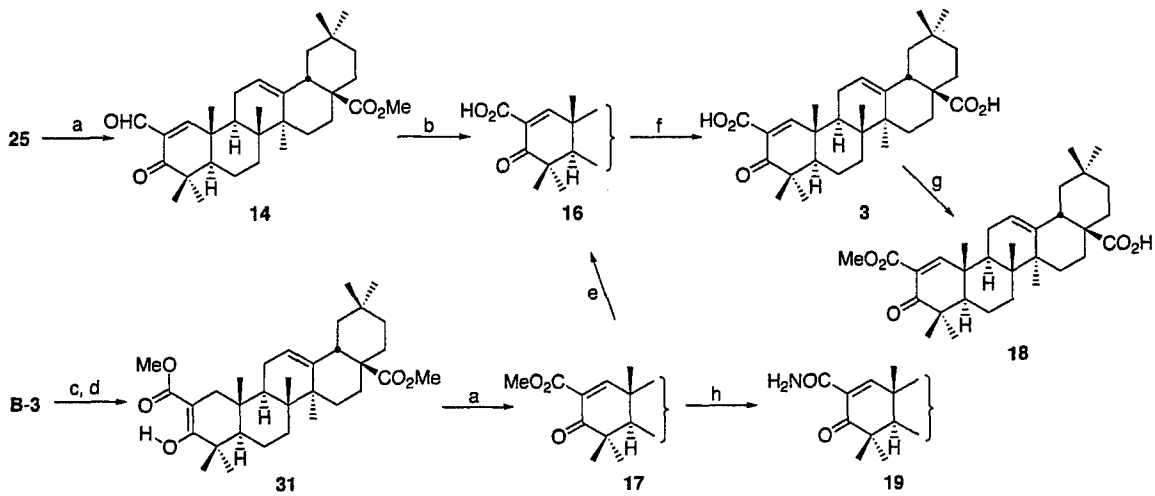
(1) In the A ring, a 1-en-3-one functionality is important for significant activity. The lead compound **B-15** is much more potent than the C-3 ketone **B-1** and the

Scheme 4[a]



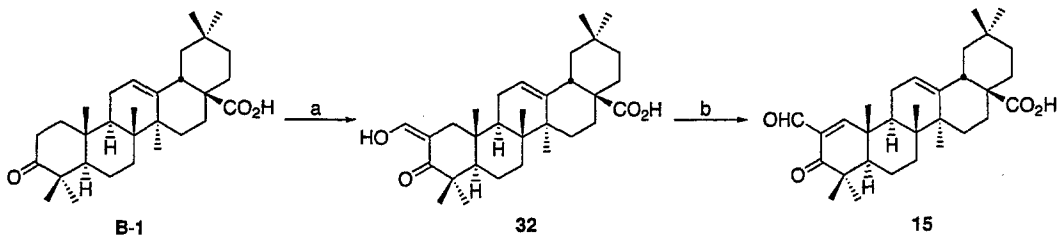
[a] Reagents: (a) HCO_2Et, NaOMe, PhH; (b) $NH_2OH{\cdot}HCl$, aq EtOH; (c) NaOMe, Et_2O, MeOH; (d) DDQ, PhH; (e) LiI, DMF.

Scheme 5[a]



[a] Reagents: (a) PhSeCl, pyr, CH_2Cl_2; 30% H_2O_2, CH_2Cl_2; (b) Jones; (c) Stiles' reagent, DMF; (d) CH_2N_2, Et_2O, THF; (e) KOH, aq MeOH; (f) LiI, DMF; (g) H_2SO_4, MeOH; (h) NH_3, MeOH.

Scheme 6[a]



[a] Reagents: (a) HCO_2Et, NaOMe, THF; (b) PhSeCl, pyr, CH_2Cl_2; 30% H_2O_2, CH_2Cl_2.

C-3 alcohol **1** (oleanolic acid). Also, the ursane derivative **B-16** is more potent than the C-3 alcohol **2** (ursolic acid).

(2) A correlation between Taft's σ^* values of substituents at C-2 and biological activity is not observed. This result shows that the activity does not depend on the strength of electron-withdrawing effect of a substituent at C-2.

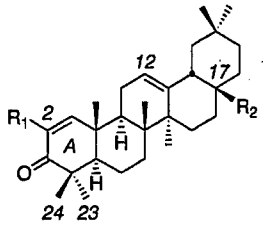
(3) Carboxyl, methoxycarbonyl, and nitrile groups at C-2 enhance activity. Compounds **3**, **10**, **11**, **16**, and **17** are about 10−100 times more potent than **B-15**. In particular, **3** showed the highest activity (IC_{50} = 0.07 μM) in this series of compounds. The potency of **3** was similar to that of hydrocortisone (IC_{50} = 0.01 μM).

(4) Hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decrease activity. Compounds **4**−**9** and **19** are much less potent than **B-15**.

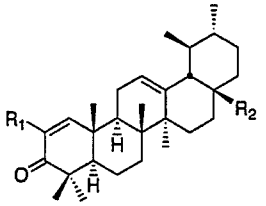
(5) A formyl group does not confer activity but only toxicity.

(6) 23,24-Dimethyl groups are important for signifi-

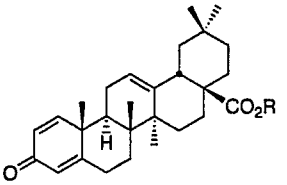
Table 2. Activity of Olean- and Urs-12-ene Triterpenoids with Various 1-En-3-one Functionalities



3-oxooleana-1,12-diene



3-oxoursa-1,12-diene



20: R = Me
21: R = H

compd	skeleton[a]	R_1 at C-2	R_2 at C-17	Taft's σ^* value of R_1	formula	analyses[b]	activity[c] IC_{50} (μM)
B-13	O	H	CO_2Me		$C_{31}H_{46}O_3$	ref 9	31
B-15	O	H	CO_2H		$C_{30}H_{44}O_3 \cdot 3/4H_2O$	C, H	5.6
20	D	H	CO_2Me		$C_{29}H_{40}O_3 \cdot 1/4H_2O$	C, H	>40
21	D	H	CO_2H		$C_{28}H_{38}O_3 \cdot 1/3H_2O$	C, H	13
B-16	U	H	CO_2H		$C_{30}H_{44}O_3$	ref 14	13
5	O	OH	CO_2H	1.34	$C_{30}H_{44}O_4 \cdot 1/2H_2O$	C, H	27
19	O	$CONH_2$	CO_2Me	1.68	$C_{32}H_{47}O_4N \cdot 3/4H_2O$	C, H, N	14
4	O	OMe	CO_2H	1.81	$C_{31}H_{46}O_4 \cdot 1/2H_2O$	C, H	30
17	O	CO_2Me	CO_2Me	2.00	$C_{33}H_{48}O_5$	C, H	0.9
18	O	CO_2Me	CO_2H	2.00	$C_{32}H_{46}O_5$	C, H	2.2
16	O	CO_2H	CO_2Me	2.08	$C_{32}H_{46}O_5 \cdot 1/2H_2O$	C, H	0.8
3	O	CO_2H	CO_2H	2.08	$C_{31}H_{44}O_5$	C, H	0.07
14	O	CHO	CO_2Me	2.15	$C_{32}H_{46}O_4$	C, H	toxic[d]
15	O	CHO	CO_2H	2.15	$C_{31}H_{44}O_4 \cdot 1/2H_2O$	C, H	toxic[d]
8	O	Br	CO_2Me	2.84	$C_{31}H_{45}O_3Br$	C, H	>40
9	O	Br	CO_2H	2.84	$C_{30}H_{43}O_3Br \cdot H_2O$	C, H	7.3
6	O	Cl	CO_2Me	2.96	$C_{31}H_{45}O_3Cl$	C, H	>40
7	O	Cl	CO_2H	2.96	$C_{30}H_{43}O_3Cl \cdot 1/4H_2O$	C, H	>40
10	O	CN	CO_2Me	3.30	$C_{32}H_{45}O_3N \cdot 1/4H_2O$	C, H, N	0.7
11	O	CN	CO_2H	3.30	$C_{31}H_{43}O_3N \cdot 1/2H_2O$	C, H, N	0.6
12	U	CN	CO_2Me	3.30	$C_{32}H_{45}O_3N \cdot 3/4H_2O$	C, H, N	5.1
13	U	CN	CO_2H	3.30	$C_{31}H_{43}O_3N \cdot H_2O$	C, H, N	6.2
B-1		oleanonic acid			$C_{30}H_{46}O_3$	ref 10	37
1		oleanolic acid			$C_{30}H_{48}O_3$	ref 10	>40
2		ursolic acid			$C_{30}H_{48}O_3$	ref 15	toxic[e]
		hydrocortisone					0.01

[a] O, 3-oxooleana-1,12-diene; D, 23,24-dinor-3-oxooleana-1,4,12-triene; U, 3-oxoursa-1,12-diene. [b] C, H, and N analyses were within ±0.4% of the theoretical values. [c] Details of the evaluation method are described in the Experimental Section. IC_{50} values of **3** and hydrocortisone were determined in the range of 0.1 pM−1 μM (10-fold dilutions). The other compounds were assayed in the range of 0.01−40 μM (4-fold dilutions). Values are an average of two separate experiments. [d] Compounds **14** and **15** were toxic to cells above 1 μM and were not active below 1 μM. [e] Ursolic acid (**2**) was toxic to cells above 10 μM and was not active below 10 μM.

cant activity. **B-15** is more potent than 23,24-dinor-olean-1-en-3-one derivative **21**.

(7) The oleanane skeleton is more potent than the ursane skeleton. **B-15**, **10**, and **11** are more potent than **B-16**, **12**, and **13**, respectively.

(8) The role of methoxycarbonyl and carboxyl groups at C-17 is ambiguous. In some analogues, the carboxyl group is more potent than the methoxycarbonyl group: acids **B-15**, **3**, **9**, and **21** are more potent than esters **B-13**, **16**, **8**, and **20**, respectively. For other analogues, the carboxyl and methoxycarbonyl groups show similar activity: acids **11** and **13** show similar activity to esters **10** and **12**, respectively. Lastly, acid **18** is less potent than ester **17**.

The inhibitory activity of new triterpenoids **3** and **11** was not blocked by the glucocorticoid antagonist, RU-486,[27] which reverses the action of hydrocortisone (see Figure 1). These data strongly suggest that the actions of these triterpenoids on the iNOS system are not mediated by their interaction with the glucocorticoid receptor.

On the basis of these structure−activity relationships, further lead optimization is in progress. Further biological evaluation and studies on the mechanism of action of **3** are also in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. UV and IR spectra were recorded on a Hewlett-Packard 8451A UV/VIS spectrophotometer and a Perkin-Elmer 600 series FTIR spectrophotometer, respectively. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 Fourier transform spectrometer. The chemical shifts are reported in δ (ppm) using the δ 7.27 signal of $CHCl_3$ (^1H NMR) and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as internal standards. Low-resolution mass spectra and high-resolution MS data were obtained on a Micromass 70-VSE unless otherwise stated. Elemental microanalysis was performed by Atlantic Microlab Inc. TLC and preparative TLC (prep-TLC) were performed with Merck precoated TLC plates silica gel 60 F_{254}. Flash column chromatography was done with Select Scientific silica gel (230−400 mesh). The standard work up method was as follows: an organic extract was washed with saturated aqueous $NaHCO_3$ solution (three times) followed by saturated aqueous NaCl solution (three times), then dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated in vacuo.

Methyl 3-Oxooleana-1,12-dien-28-oate (B-13).[9] A solution of methyl oleanonate (**B-3**)[10] (2.00 g, 4.27 mmol) and phenylselenenyl chloride (98%) (1.00 g, 5.12 mmol) in EtOAc (85 mL) was stirred at room temperature for 3 h. To the stirred

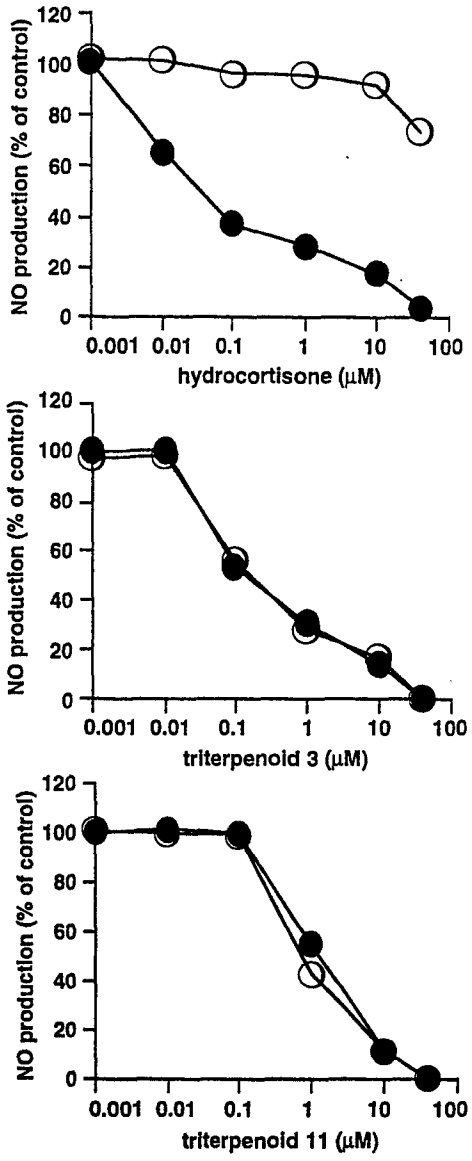


Figure 1. Blockage by glucocorticoid antagonist RU486 of hydrocortisone-inhibited NO production but not of triterpenoid (**3** and **11**) inhibited NO production in primary mouse macrophages. Macrophage cells were incubated with IFN-γ (20 ng/mL) together with hydrocortisone or triterpenoids without RU486 (●); in some cases RU486 (1 μM) was added simultaneously to both hydrocortisone- and triterpenoid-treated cell wells (○). RU486 itself does not interfere with NO production at the concentration tested.

mixture, saturated aqueous $NaHCO_3$ solution was added. After most of the aqueous layer was removed, pyridine (844 mg, 10.7 mmol) and *m*-chloroperbenzoic acid (50–60%) (3.68 g, 10.7 mmol) were added to the organic layer. The mixture was stirred at room temperature for 1 h. The mixture was washed with 5% aqueous NaOH solution (three times), saturated aqueous NH_4Cl solution (three times), and saturated aqueous NaCl solution (three times); dried over anhydrous $MgSO_4$; and filtered. The filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to give **B-13** as a crystalline solid (1.23 g, 62%): mp 159–161 °C; $[\alpha]^{25}_D$ +103° (*c* 0.64, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 230 (3.92) nm. IR (KBr): 2946, 2867, 1728, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.04 (1H, d, J = 10.1 Hz), 5.81 (1H, d, J = 10.1 Hz), 5.36 (1H, t, J = 3.7 Hz), 3.64, (3H, s), 2.90 (1H, dd, J = 4.6, 13.9 Hz), 1.17 (3H, s), 1.16 (6H, s), 1.10, 0.94, 0.91, 0.83 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.5, 178.4, 159.3, 144.5, 125.2, 121.9, 53.6, 51.8, 47.0, 45.9, 44.7, 42.2, 42.0, 41.7, 40.3, 39.7, 34.1, 33.3, 32.7, 32.5, 30.9, 28.0, 27.9, 26.0, 23.8, 23.5, 23.2, 21.8, 19.1, 18.8, 17.5. EIMS (70 eV) *m/z*: 466 $[M]^+$ (73), 451 (11), 407 (31), 262 (57), 203 (100). HREIMS: Calcd for $C_{31}H_{46}O_3$: 466.3447. Found: 466.3446.

Methyl 3-Oxoursa-1,12-dien-28-oate (B-14). **B-14** was prepared from methyl ursonate (**B-4**)[15] according to the same method as for **B-13** to give an amorphous solid (66%): $[\alpha]^{26}_D$ +93° (*c* 0.77, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 232 (3.95) nm. IR (KBr): 2974, 2935, 2871, 1725, 1669 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.06 (1H, d, J = 10.1 Hz), 5.81 (1H, d, J = 10.1 Hz), 5.33 (1H, t, J = 3.8 Hz), 3.63 (3H, s), 2.28 (1H, d, J = 11.5 Hz), 1.17, 1.15 (each 3H, s), 1.10 (6H, s), 0.95 (3H, d, J = 5.4 Hz), 0.87 (3H, d, J = 6.3 Hz), 0.85 (3H, s). ^{13}C NMR ($CDCl_3$): δ 205.5, 178.2, 159.5, 139.0, 125.2, 125.0, 53.7, 53.3, 51.7, 48.4, 44.7, 42.6, 41.9, 40.5, 39.5, 39.2, 39.1, 36.8, 33.0, 30.8, 28.2, 28.1, 24.4, 23.7, 23.5, 21.8, 21.4, 19.1, 19.0, 17.7, 17.2. EIMS (70 eV) *m/z*: 466 $[M]^+$ (14), 406 (12), 262 (74), 203 (100). HREIMS: Calcd for $C_{31}H_{46}O_3$: 466.3447. Found: 466.3442.

3-Oxooleana-1,12-dien-28-oic Acid (B-15). A mixture of **B-13** (100 mg, 0.21 mmol) and LiI (500 mg) in dry DMF (2 mL) was heated under reflux for 6 h. The mixture was acidified with 5% aqueous HCl solution and then extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) three times. The extract was worked up according to the standard method to give a solid (110 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (2:1)] to give **B-15** as an amorphous solid (82 mg, 85%): $[\alpha]^{26}_D$ +103° (*c* 0.45, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 230 (3.75) nm. IR (KBr): 2941, 2866, 1732, 1695, 1671 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.04 (1H, d, J = 10.2 Hz), 5.81 (1H, d, J = 10.2 Hz), 5.35 (1H, t, J = 3.3 Hz), 2.86 (1H, dd, J = 4.2, 13.4 Hz), 1.16, 1.152, 1.147, 1.07, 0.94, 0.91, 0.84 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.5, 184.5, 159.2, 144.2, 125.3, 122.1, 53.5, 46.8, 45.8, 44.7, 42.1, 41.9, 41.3, 40.2, 39.7, 34.0, 33.3, 32.6, 32.5, 30.9, 28.0, 27.8, 26.0, 23.7, 23.5, 23.0, 21.8, 19.0, 18.9, 17.7. EIMS (70 eV) *m/z*: 452 $[M]^+$ (8.8), 437 (3.8), 406 (6.8), 248 (80), 233 (14), 203 (100). HREIMS: Calcd for $C_{30}H_{44}O_3$: 452.3290. Found: 452.3289. Anal. (Table 2).

3-Oxoursa-1,12-dien-28-oic Acid (B-16).[14] **B-16** was prepared from **B-14** according to the same method as for **B-15** to give an amorphous solid (88%): $[\alpha]^{26}_D$ +91° (*c* 0.84, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 230 (3.99) nm. IR (KBr): 3306, 2973, 2930, 2870, 1729, 1695, 1669 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.07 (1H, d, J = 10.1 Hz), 5.82 (1H, d, J = 10.1 Hz), 5.33 (1H, t, J = 3.7 Hz), 2.24 (1H, d, J = 11.2 Hz), 1.18, 1.16, 1.11, 1.09 (each 3H, s), 0.96 (3H, d, J = 6.1 Hz), 0.88 (3H, d, J = 6.4 Hz), 0.88 (3H, s). ^{13}C NMR ($CDCl_3$): δ 205.5, 183.9, 159.4, 138.8, 125.3, 53.6, 52.9, 48.3, 44.7, 42.5, 41.9, 40.5, 39.6, 39.2, 39.0, 36.8, 32.9, 30.8, 28.2, 28.1, 24.2, 23.7, 23.4, 21.8, 21.3, 19.0, 17.8, 17.2. FABMS (NBA) *m/z*: 453 $[M + H]^+$ (100) (by a Micromass ZAB-SE). HRFABMS: Calcd for $C_{30}H_{44}O_3$ + H: 453.3369. Found: 453.3335 (by a Micromass 70-SE-4F).

2-Carboxy-3-oxooleana-1,12-dien-28-oic Acid (3). A mixture of **16** (109 mg, 0.21 mmol) and LiI (520 mg) in dry DMF (1.5 mL) was heated under reflux for 1 h. After 5% aqueous HCl solution was added, the acidic mixture was extracted with EtOAc three times. The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a residue (108 mg). The residue was subjected to flash column chromatography [CH_2Cl_2–MeOH (15:1) followed by CH_2Cl_2–MeOH (10:1)] to afford **3** as a crystalline solid (61 mg, 58%): mp >250 °C dec; $[\alpha]^{26}_D$ +81° (*c* 0.53, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 234 (3.88) nm. IR (KBr): 3389, 2943, 2872, 1752, 1696, 1637 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.43 (1H, s), 5.37 (1H, t, J = 3.5 Hz), 2.87 (1H, dd, J = 3.8, 13.9 Hz), 1.25, 1.22, 1.18, 1.15, 0.95, 0.93, 0.88 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 209.0, 183.9, 173.2, 165.2, 144.2, 123.4, 121.7, 52.4, 46.8, 45.7, 45.5, 42.3, 41.4, 41.1, 40.6, 40.4, 34.0, 33.2, 32.5, 32.3, 30.9, 28.4, 27.8, 26.0, 23.7, 23.5, 23.0, 22.0, 19.0, 18.4, 17.8. EIMS (70 eV) *m/z*: 496 $[M]^+$ (3.0), 478 (3.4), 452 (7.6), 248 (56), 231 (35), 203 (100). HREIMS: Calcd for $C_{31}H_{44}O_5$: 496.3189. Found: 496.3196. Anal. (Table 2).

2-Hydroxy-3-oxooleana-1,12-dien-28-oic Acid (4). **4** was prepared from **24** according to the same method as for **B-15**

except that the reaction time was 2 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (4:1)] to give **4** as an amorphous solid (18%): $[\alpha]^{25}_D$ +99° (*c* 0.46, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 272 (3.71) nm. IR (KBr): 3434, 2938, 1698, 1667, 1649 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.35 (1H, s), 5.96 (1H, brs), 5.34 (1H, t, J = 3.5 Hz), 2.86 (1H, dd, J = 3.8, 13.9 Hz), 1.23, 1.22, 1.14, 1.11, 0.94, 0.92, 0.83 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 201.2, 184.0, 144.1, 143.9, 128.4, 122.3, 54.0, 46.8, 45.8, 44.1, 43.3, 42.2, 41.3, 40.2, 38.7, 34.0, 33.3, 32.6, 30.9, 27.8, 27.4, 26.1, 23.8, 23.6, 23.0, 22.0, 19.9, 18.9, 17.7. EIMS (70 eV) *m/z*: 468 $[M]^+$ (3.2), 248 (13), 203 (23), 149 (42), 84 (100). HREIMS: Calcd for $C_{30}H_{44}O_4$: 468.3240. Found: 468.3222. Anal. (Table 2).

2-Methoxy-3-oxooleana-1,12-dien-28-oic Acid (5). A mixture of **23** (230 mg, 0.46 mmol) and LiI (1045 mg) in dry DMF (3.5 mL) was heated under reflux for 4 h. The reaction mixture was worked up according to the same method as for **B-15** to give a solid (230 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1), then hexanes–EtOAc (1:1)] to give **24** (35 mg; 16%, 23% based on recovered **23**), **23** (74 mg), **4** (27 mg; 12%, 18% based on recovered **23**), and **5** as an amorphous solid (63 mg; 28%, 41% based on recovered **23**): $[\alpha]^{26}_D$ +96° (*c* 0.29, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 266 (3.84) nm. IR (KBr): 3307, 2947, 2862, 1732, 1693, 1622 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.96 (1H, s), 5.36 (1H, t, J = 3.3 Hz), 3.56 (3H, s), 2.87 (1H, dd, J = 4.2, 13.9 Hz), 1.17 (9H, s), 1.11, 0.94, 0.91, 0.84 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.0, 184.4, 149.1, 144.4, 126.1, 122.1, 55.0, 53.2, 46.8, 45.9, 45.4, 43.3, 42.2, 41.3, 40.2, 38.5, 34.0, 33.3, 32.5, 30.9, 28.6, 27.8, 26.1, 23.8, 23.0, 22.0, 20.4, 19.2, 17.6. EIMS (70 eV) *m/z*: 482 $[M]^+$ (11), 415 (6.5), 245 (18), 203 (33), 157 (100). HREIMS: Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3375. Anal. (Table 2).

Methyl 2-Chloro-3-oxooleana-1,12-dien-28-oate (6). A solution of **22** (99 mg, 0.21 mmol) in AcOH including 1 M HCl (2.5 mL) and $CHCl_3$ (2.5 mL) was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2. After it was washed with water three times, it was worked up according to the standard method to give a solid (96 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (6:1)] to afford **6** as an amorphous solid (84 mg, 81%): $[\alpha]^{26}_D$ +98° (*c* 0.26, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 250 (3.91) nm. IR (KBr): 2943, 2866, 1727, 1689 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.22 (1H, s), 5.34 (1H, t, J = 3.5 Hz), 3.62 (3H, s), 2.89 (1H, dd, J = 4.2, 13.7 Hz), 1.203, 1.197 (each 3H, s), 1.14 (6H, s), 0.93, 0.90, 0.80 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 197.4, 178.3, 155.0, 144.5, 129.8, 121.5, 53.3, 51.8, 46.9, 46.3, 45.8, 42.2, 42.1, 41.64, 41.57, 40.3, 34.0, 33.3, 32.4, 30.9, 28.4, 27.8, 26.0, 23.8, 23.5, 23.1, 22.1, 19.1, 18.8, 17.5. EIMS (70 eV) *m/z*: 500 $[M]^+$ (21), 262 (27), 247 (96), 203 (100). HREIMS: Calcd for $C_{31}H_{45}O_3Cl$: 500.3057. Found: 500.3060. Anal. (Table 2).

2-Chloro-3-oxooleana-1,12-dien-28-oic Acid (7). **7** was prepared from **6** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (4:1) followed by hexanes–EtOAc (3:1)] to give **7** as an amorphous solid (77%): $[\alpha]^{26}_D$ +88° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 252 (3.20) nm. IR (KBr): 3297, 2943, 2870, 1733, 1691, 1601 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.23 (1H, s), 5.35 (1H, t, J = 3.3 Hz), 2.86 (1H, dd, J = 4.3, 13.8 Hz), 1.22, 1.21, 1.16, 1.13, 0.94, 0.92, 0.84 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 197,4, 184.4, 154.9, 144.3, 129.9, 121.8, 53.2, 46.8, 46.4, 45.8, 42.21, 42.16, 41.6, 41.3, 40.3, 34.0, 33.3, 32.5, 32.4, 30.9, 28.5, 27.8, 26.0, 23.7, 23.5, 23.0, 22.1, 19.0, 18.9, 17.7. EIMS (70 eV) *m/z*: 486 $[M]^+$ (25), 248 (100), 203 (96). HREIMS: Calcd for $C_{30}H_{43}O_3Cl$: 486.2901. Found: 486.2898. Anal. (Table 2).

Methyl 2-Bromo-3-oxooleana-1,12-dien-28-oate (8). A solution of **22** (220 mg, 0.46 mmol) in AcOH including 1 M HBr (4.9 mL) and $CHCl_3$ (6.1 mL) was stirred at room temperature for 1 h. The mixture was diluted with CH_2Cl_2. After it was washed with water three times, it was worked up according to the standard method to give a solid (260 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (6:1)] to afford **8** as an amorphous solid (238 mg, 96%): $[\alpha]^{26}_D$ +88° (*c* 0.51, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 260 (3.69) nm. IR (KBr): 2943, 2870, 1733, 1691, 1601 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.49 (1H, s), 5.35 (1H, t, J = 3.5 Hz), 3.63 (3H, s), 2.90 (1H, dd, J = 4.0, 13.8 Hz), 1.20, 1.15 (each 6H, s), 0.94, 0.91, 0.81 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 197.3, 178.3, 159.5, 144.6, 121.8, 121.5, 53.3, 51.8, 46.9, 46.5, 45.8, 43.1, 42.3, 42.1, 41.7, 40.3, 34.0, 33.3, 32.4, 30.9, 28.7, 27.8, 26.0, 23.8, 23.6, 23.2, 22.3, 19.1, 18.7, 17.5. EIMS (70 eV) *m/z*: 546 (5.0) and 544 (5.2) $[M]^+$, 262 (8.5), 203 (24), 118 (100), 116 (100). HREIMS: Calcd for $C_{31}H_{45}O_3Br$: 544.2552. Found: 544.2553. Anal. (Table 2).

2-Bromo-3-oxooleana-1,12-dien-28-oic Acid (9). **9** was prepared from **8** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (4:1) followed by hexanes–EtOAc (3:1)] to give **9** as an amorphous solid (76%): $[\alpha]^{26}_D$ +82° (*c* 0.31, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 260 (3.52) nm. IR (KBr): 3434, 2939, 2870, 1727, 1686, 1601 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.49 (1H, s), 5.35 (1H, t, J = 3.4 Hz), 2.86 (1H, dd, J = 4.2, 13.7 Hz), 1.21 (6H, s), 1.16, 1.14, 0.94, 0.92, 0.83 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 197.2, 184.4, 159.3, 144.3, 121.84, 121.79, 53.3, 46.8, 46.5, 45.8, 43.1, 42.2, 42.0, 41.3, 40.3, 34.0, 33.2, 32.5, 32.4, 30.9, 28.7, 27.8, 26.0, 23.7, 23.5, 23.0, 22.2, 19.1, 18.7, 17.7. EIMS (70 eV) *m/z*: 532 (13) and 530 (14) $[M]^+$, 285 (5.6), 283 (6.2), 248 (100), 235 (10), 233 (11), 203 (84). HREIMS: Calcd for $C_{30}H_{43}O_3Br$: 530.2396. Found: 530.2383. Anal. (Table 2).

Methyl 2-Cyano-3-oxooleana-1,12-dien-28-oate (10). A solution of **27** (141 mg, 0.28 mmol) and DDQ (98%) (79 mg, 0.34 mmol) in benzene (10 mL) was heated under reflux for 4 h. After insoluble matter was removed by filtration, the filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [benzene–acetone (20:1)] to give a crystalline solid (123 mg, 88%): mp 201–202 °C; $[\alpha]^{26}_D$ +67° (*c* 0.53, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (3.65) nm. IR (KBr): 2945, 2874, 2232, 1724, 1686 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.75 (1H, s), 5.36 (1H, t, J = 3.5 Hz), 3.64 (3H, s), 2.91 (1H, dd, J = 3.9, 13.9 Hz), 1.22, 1.21, 1.15, 1.14, 0.94, 0.92, 0.83 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 198.3, 178.3, 170.2, 144.8, 121.1, 115.2, 114.0, 52.8, 51.8, 46.9, 45.8, 45.1, 42.3, 41.7, 41.3, 40.8, 40.5, 34.0, 33.3, 32.4, 32.3, 30.9, 27.9, 27.8, 26.0, 23.8, 23.4, 23.1, 21.8, 18.9, 18.1, 17.6. EIMS (70 eV) *m/z*: 491 $[M]^+$ (35), 459 (13), 432 (27), 262 (22), 247 (24), 203 (100). HREIMS: Calcd for $C_{32}H_{45}O_3N$: 491.3399. Found: 491.3391. Anal. (Table 2).

2-Cyano-3-oxooleana-1,12-dien-28-oic Acid (11). **11** was prepared from **10** according to the same method as for **B-15** except that the reaction time was 3 h. The reaction mixture was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1), then hexanes–EtOAc (1:1)] to give **11** as an amorphous solid (71%, 91% based on recovered **10**): $[\alpha]^{26}_D$ +61° (*c* 0.66, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 238 (3.87) nm. IR (KBr): 3387, 2947, 2870, 2233, 1729, 1691, 1609 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.75 (1H, s), 5.35 (1H, t, J = 3.3 Hz), 2.86 (1H, dd, J = 4.0, 13.6 Hz), 1.22, 1.21, 1.15, 1.12, 0.94, 0.92, 0.85 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 198.2, 184.3, 170.1, 144.4, 121.4, 115.1, 114.1, 52.7, 46.8, 45.7, 45.0, 42.2, 41.3, 40.8, 40.5, 33.9, 33.2, 32.5, 32.2, 30.9, 27.9, 27.7, 25.9, 23.7, 23.4, 22.9, 21.8, 18.9, 18.1, 17.7. EIMS (70 eV) *m/z*: 477 $[M]^+$ (18), 462 (5.6), 431 (16), 416 (10), 248 (76), 235 (25), 203 (100). HREIMS: Calcd for $C_{31}H_{43}O_3N$: 477.3243. Found: 477.3240. Anal. (Table 2).

Methyl 2-Cyano-3-oxoursa-1,12-dien-28-oate (12). **12** was prepared from **30** according to the same method as for **10** to give an amorphous solid (62%): $[\alpha]^{26}_D$ +53° (*c* 0.35, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (3.74) nm. IR (KBr): 2973, 2926, 2870, 2229, 1723, 1686 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.77 (1H, s), 5.33 (1H, t, J = 3.7 Hz), 3.62 (3H, s), 2.29 (1H, d, J = 11.2 Hz), 1.23, 1.21, 1.14, 1.11 (each 3H, s), 0.96, 0.88 (each 3H, d, J = 6.3 Hz), 0.86 (3H, s). ^{13}C NMR ($CDCl_3$): δ 198.3, 178.1, 170.4, 139.3, 124.2, 115.2, 114.0, 53.2, 52.8, 51.7, 48.3, 45.1, 42.7, 41.2, 40.70, 40.65, 39.1, 39.0, 36.7, 32.6, 30.8, 28.1, 28.0, 24.3, 23.6, 23.4, 21.8, 21.3, 18.9, 18.2, 17.8, 17.2. EIMS (70

eV) *m/z*: 491 $[M]^+$ (38), 431 (35), 262 (46), 249 (82), 203 (65), 84 (100). HREIMS: Calcd for $C_{32}H_{45}O_3N$: 491.3399. Found: 491.3395. Anal. (Table 2).

2-Cyano-3-oxoursa-1,12-dien-28-oic Acid (13). **13** was prepared from **12** according to the same method as for **B-15** except that the reaction time was 4 h. The reaction mixture was subjected to prep-TLC [hexanes−EtOAc (1.5:1)] to give **13** as an amorphous solid (74%): $[\alpha]^{26}_D$ +48° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 238 (3.86) nm. IR (KBr): 3417, 2973, 2926, 2870, 2233, 1731, 1689 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.77 (1H, s), 5.31 (1H, t, J = 3.2 Hz), 2.24 (1H, d, J = 11.0 Hz), 1.22, 1.20, 1.12, 1.11 (each 3H, s), 0.95, 0.88 (each 3H, d, J = 5.7 Hz), 0.87 (3H, s). ^{13}C NMR ($CDCl_3$): δ 198.2, 184.2, 170.2, 139.0, 124.4, 115.1, 114.1, 52.8, 52.7, 48.2, 45.0, 42.6, 41.2, 40.68, 40.65, 39.1, 39.0, 36.7, 32.5, 30.7, 28.1, 28.0, 24.1, 23.6, 23.3, 21.8, 21.3, 18.9, 18.2, 17.7, 17.2. EIMS (70 eV) *m/z*: 477 $[M]^+$ (22), 431 (23), 248 (100), 203 (48). HREIMS: Calcd for $C_{31}H_{43}O_3N$: 477.3243. Found: 477.3240. Anal. (Table 2).

Methyl 2-Formyl-3-oxooleana-1,12-dien-28-oate (14). To a stirred solution of phenylselenenyl chloride (98%) (161 mg, 0.82 mmol) in CH_2Cl_2 (7.2 mL) was added a solution of pyridine (75 mg, 0.95 mmol) in CH_2Cl_2 (1.0 mL) in an ice bath. After 15 min, a solution of **25** (204 mg, 0.41 mmol) in CH_2Cl_2 (2.0 mL) was added, and the mixture was stirred an additional 1 h. After the mixture was washed with 10% aqueous HCl solution (3 mL) twice, 30% H_2O_2 (0.4 mL) was added to the stirred mixture in the ice bath. After an additional 40 min, the mixture was worked up according to the standard method to give a solid (199 mg). The solid was subjected to flash column chromatography [hexanes−EtOAc (5:1)] to afford **25** (20 mg) and **14** as an amorphous solid (144 mg; 71%, 79% based on recovered **25**): $[\alpha]^{26}_D$ +12° (*c* 0.60, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 238 (3.85) nm. IR (KBr): 2946, 2867, 1724, 1703, 1673, 1610 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.00 (1H, s), 7.79 (1H, s), 5.37 (1H, t, J = 3.6 Hz), 3.63 (3H, s), 2.90 (1H, dd, J = 4.2, 13.9 Hz), 1.18, 1.17, 1.16, 1.14, 0.94, 0.91, 0.85 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 203.7, 190.7, 178.3, 165.2, 144.5, 131.2, 121.6, 52.8, 51.8, 47.0, 45.8, 45.1, 42.3, 41.7, 41.3, 40.5, 39.8, 34.0, 33.3, 32.44, 32.38, 30.9, 28.2, 27.8, 26.0, 23.8, 23.5, 23.2, 21.7, 19.2, 18.2, 17.6. EIMS (70 eV) *m/z*: 494 $[M]^+$ (95), 435 (87), 262 (40), 203 (100). HREIMS: Calcd for $C_{32}H_{46}O_4$: 494.3396. Found: 494.3398. Anal. (Table 2).

2-Formyl-3-oxooleana-1,12-dien-28-oic Acid (15). **15** was prepared from **32** according to the same method as for **14**. The reaction mixture was subjected to flash column chromatography [hexanes−EtOAc (3:1) followed by hexanes−EtOAc (2:1)] to give **15** as an amorphous solid (71%, 84% based on recovered **32**): $[\alpha]^{26}_D$ +26° (*c* 0.95, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (3.82) nm. IR (KBr): 2948, 2866, 1725, 1701, 1674, 1608 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.00 (1H, s), 7.79 (1H, s), 5.36 (1H, t, J = 3.3 Hz), 2.86 (1H, dd, J = 3.8, 13.9 Hz), 1.18, 1.17, 1.15, 1.14, 0.94, 0.92, 0.87 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 203.7, 190.7, 184.3, 165.0, 144.2, 131.2, 121.8, 52.8, 46.8, 45.7, 45.1, 42.3, 41.4, 41.3, 40.5, 39.8, 34.0, 33.2, 32.5, 32.3, 30.9, 28.2, 27.8, 26.0, 23.7, 23.5, 23.0, 21.6, 19.2, 18.2, 17.8. EIMS (70 eV) *m/z*: 480 $[M]^+$ (5.5), 434 (3.1), 419 (3.4), 248 (56), 233 (27), 203 (100). HREIMS: Calcd for $C_{31}H_{44}O_4$: 480.3240. Found: 480.3237. Anal. (Table 2).

Methyl 2-Carboxy-3-oxooleana-1,12-dien-28-oate (16). **(1) From 14**: To a solution of **14** (357 mg, 0.72 mmol) in acetone (71 mL) was added Jones reagent (0.5 mL) dropwise in an ice bath. The mixture was stirred in the ice bath for 20 min. After excess Jones reagent was decomposed with MeOH, the acetone was evaporated in vacuo. After water was added to the resultant mixture, the aqueous mixture was extracted with EtOAc three times. The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a residue (294 mg). The residue was subjected to flash column chromatography [hexanes−EtOAc (1:1) followed by EtOAc] to afford **14** (89 mg) and **16** as a crystalline solid (109 mg; 30%, 39% based on recovered **14**): mp 230−231 °C; $[\alpha]^{26}_D$ +85° (*c* 0.61, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 234 (3.78) nm. IR (KBr): 3436, 2946, 2876, 1756, 1722, 1633 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.43 (1H, s), 5.36 (1H, t, J = 3.5 Hz), 3.64 (3H, s), 2.90 (1H, dd, J = 3.9, 13.7 Hz), 1.24, 1.21, 1.19, 1.13, 0.94, 0.91, 0.85 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 209.2, 178.4, 173.4, 165.2, 144.5, 123.3, 121.4, 52.4, 51.8, 47.0, 45.7, 45.5, 42.3, 41.7, 41.1, 40.6, 40.4, 34.0, 33.3, 32.4, 32.3, 30.9, 28.3, 27.8, 26.0, 23.8, 23.5, 23.1, 22.0, 19.0, 18.3, 17.7. EIMS (70 eV) *m/z*: 510 $[M]^+$ (16), 492 (15), 451 (14), 433 (14), 262 (27), 203 (100). HREIMS: Calcd for $C_{32}H_{46}O_5$: 510.3345. Found: 510.3347. Anal. (Table 2).

(2) From 17: A solution of **17** (500 mg, 0.95 mmol) in MeOH (29 mL) and aqueous KOH solution (KOH, 2.9 g; water, 10 mL) was heated under reflux for 15 min. After removal of MeOH in vacuo, the mixture was acidified with 5% aqueous HCl solution. It was extracted with EtOAc (three times). The extract was washed with water and saturated aqueous NaCl solution (each three times), dried over $MgSO_4$, and filtered. The filtrate gave **16** as a crystalline solid (470 mg, 97%). It was used for the next reaction without further purification.

Methyl 2-Methoxycarbonyl-3-oxooleana-1,12-dien-28-oate (17). **17** was prepared from **31** by the similar method as for **14**. The reaction mixture was subjected to flash column chromatography [hexanes−EtOAc (4:1)] to give **17** as an amorphous solid (83%, 90% based on recovered **31**): $[\alpha]^{26}_D$ +63° (*c* 0.78, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 230 (3.97) nm. IR (KBr): 2947, 2866, 1727, 1684, 1624 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.73 (1H, s), 5.37 (1H, t, J = 3.5 Hz), 3.79, 3.64 (each 3H, s), 2.90 (1H, dd, J = 3.9, 13.7 Hz), 1.16 (6H, s), 1.15, 1.12, 0.94, 0.91, 0.84 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 201.2, 178.4, 166.0, 164.3, 144.5, 129.2, 121.7, 52.7, 52.4, 51.8, 47.0, 45.9, 45.8, 42.3, 41.8, 41.5, 40.3, 39.5, 34.1, 33.3, 32.4, 32.3, 30.9, 28.7, 27.8, 25.9, 23.8, 23.6, 23.2, 21.5, 19.4, 18.0, 17.5. EIMS (70 eV) *m/z*: 524 $[M]^+$ (24), 492 (23), 465 (13), 262 (35), 203 (100). HREIMS: Calcd for $C_{33}H_{48}O_5$: 524.3502. Found: 524.3494. Anal. (Table 2).

2-Methoxycarbonyl-3-oxooleana-1,12-dien-28-oic Acid (18). A solution of **3** (52 mg, 0.10 mmol) in MeOH (5.2 mL) containing concentrated H_2SO_4 (0.15 mL) was heated under reflux for 30 min. After saturated aqueous NaCl solution was added to the mixture, it was extracted with EtOAc three times. The extract was worked up according to the standard method to give a residue (53 mg). The residue was subjected to flash column chromatography [hexanes−EtOAc (2:1)] to give **18** as an amorphous solid (42 mg, 78%): $[\alpha]^{26}_D$ +61° (*c* 0.56, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 230 (3.83) nm. IR (KBr): 3323, 2947, 2866, 1733, 1695, 1622 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.73 (1H, s), 5.37 (1H, t, J = 3.4 Hz), 3.79 (3H, s), 2.86 (1H, dd, J = 4.1, 13.7 Hz), 1.16, 1.15, 1.14, 1.12, 0.94, 0.92, 0.86 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 201.1, 184.2, 166.0, 164.2, 144.2, 129.2, 122.0, 52.7, 52.4, 46.9, 45.9, 45.8, 42.2, 41.5, 41.4, 40.3, 39.5, 34.0, 33.3, 32.5, 32.3, 30.9, 28.7, 27.8, 26.0, 23.7, 23.6, 23.0, 21.4, 19.4, 18.0, 17.7. EIMS (70 eV) *m/z*: 510 $[M]^+$ (2.6), 495 (2.0), 478 (2.5), 432 (3.0), 263 (29), 248 (58), 231 (37), 203 (100). HREIMS: Calcd for $C_{32}H_{46}O_5$: 510.3345. Found: 510.3344. Anal. (Table 2).

Methyl 2-Aminocarbonyl-3-oxooleana-1,12-dien-28-oate (19). A solution of **17** (100 mg, 0.19 mmol) in saturated ammonia MeOH (10 mL) was kept at room temperature overnight. The mixture was evaporated in vacuo to give a solid (108 mg). The solid was subjected to flash column chromatography [hexanes−EtOAc (1.5:1)] to give **19** as an amorphous solid (94 mg, 96%): $[\alpha]^{26}_D$ +77° (*c* 0.60, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 236 (3.91) nm. IR (KBr): 3413, 2943, 2866, 1727, 1689 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.45 (1H, brs), 8.27 (1H, s), 5.72 (1H, brs), 5.37 (1H, t, J = 3.4 Hz), 3.64 (3H, s), 2.90 (1H, dd, J = 4.2, 13.9 Hz), 1.17, 1.16, 1.15, 1.14, 0.94, 0.92, 0.84 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.8, 178.4, 169.0, 165.8, 144.3, 121.8, 52.2, 51.8, 47.0, 46.0, 45.7, 42.3, 41.8, 41.2, 40.4, 39.6, 34.1, 33.3, 32.5, 32.3, 30.9, 29.1, 27.8, 26.0, 23.8, 23.6, 23.2, 21.9, 19.4, 18.6, 17.6. EIMS (70 eV) *m/z*: 509 $[M]^+$ (34), 492 (23), 450 (100), 262 (19), 203 (56). HREIMS: Calcd for $C_{32}H_{47}O_4N$: 509.3505. Found: 509.3500. Anal. (Table 2).

Methyl 1α,2α-Epoxy-3-oxoolean-12-en-28-oate (22).[9] To a solution of **B-13** (223 mg, 0.48 mmol) in 2 N aqueous NaOH solution (1.7 mL) and THF (11 mL) was added a solution of

30% H_2O_2 (1.4 mL) in MeOH (2.8 mL) in an ice bath. The mixture was stirred at room temperature for 4 h. To the mixture were added saturated aqueous $NaHSO_3$ and 5% aqueous NaOH solutions, successively. After removal of THF and MeOH, the resultant mixture was acidified with 6 M aqueous HCl solution. The acidic layer was extracted with CH_2Cl_2 three times. The extract was worked up according to the standard method to give a crystalline solid (228 mg, 99%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by recrystallization from MeOH as colorless needles: mp 212–213 °C; $[\alpha]^{26}_D$ +157° (*c* 0.80, $CHCl_3$). IR (KBr): 2943, 2866, 1727, 1699 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.36 (1H, t, J = 3.3 Hz), 3.64 (3H, s), 3.50 (1H, d, J = 4.5 Hz), 3.37 (1H, d, J = 4.5 Hz), 2.90 (1H, dd, J = 4.2, 13.9 Hz), 1.21, 1.11, 1.01, 0.97, 0.94, 0.92, 0.80 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 213.0, 178.4, 144.5, 121.8, 64.1, 57.1, 51.8, 47.0, 46.3, 45.9, 45.0, 42.1, 41.7, 40.8, 39.7, 38.8, 34.1, 33.3, 32.5, 32.3, 30.9, 28.2, 28.0, 26.0, 24.0, 23.8, 23.3, 21.1, 19.1, 17.4, 15.1. EIMS (70 eV) *m/z*: 482 $[M]^+$ (7.7), 422 (13), 262 (31), 249 (11), 203 (100). HREIMS: Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3391.

Methyl 2-Methoxy-3-oxooleana-1,12-dien-28-oate (23). A mixture of **22** (300 mg, 0.62 mmol) and Na (360 mg) in MeOH (36 mL) was heated under reflux for 48 h. After removal of MeOH in vacuo, the resultant mixture was diluted with water and then acidified with 6 M aqueous HCl solution. The aqueous mixture was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) three times. The extract was worked up according to the standard method to give a solid (320 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (4:1)] to afford **22** (31 mg) and **23** as an amorphous solid (270 mg; 87%, 98% based on recovered **22**): UV (EtOH) λ_{max} (log ϵ): 266 (3.77) nm. IR (KBr): 2946, 2866, 1727, 1682, 1621 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.96 (1H, s), 5.36 (1H, t, J = 3.5 Hz), 3.64, 3.55 (each 3H, s), 2.90 (1H, dd, J = 4.1, 13.7 Hz), 1.17 (6H, s), 1.16, 1.13, 0.93, 0.90, 0.81 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.1, 178.4, 149.0, 144.6, 126.3, 121.9, 54.9, 53.2, 51.8, 47.0, 45.9, 45.4, 43.3, 42.3, 41.7, 40.2, 38.4, 34.0, 33.3, 32.6, 32.5, 30.9, 28.5, 27.8, 26.0, 23.81, 23.76, 23.2, 22.0, 20.4, 19.2, 17.4. EIMS (70 eV) *m/z*: 496 $[M]^+$ (80), 436 (21), 328 (19), 262 (36), 203 (100). HREIMS: Calcd for $C_{32}H_{48}O_4$: 496.3553. Found: 496.3544.

Methyl 2-Hydroxy-3-oxooleana-1,12-dien-28-oate (24). A suspension of **23** (100 mg, 0.20 mmol) in 3 M aqueous HCl solution (3 mL) and AcOH (3 mL) was heated under reflux for 5 h. The mixture was neutralized with saturated aqueous Na_2CO_3 solution. The mixture was extracted with CH_2Cl_2 three times. The extract was worked up according to the standard method to give a solid (90 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to afford **24** as an amorphous solid (78 mg, 81%): UV (EtOH) λ_{max} (log ϵ): 272 (3.63) nm. IR (KBr): 3426, 2939, 2870, 1725, 1667, 1648 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.35 (1H, s), 5.93 (1H, brs), 5.34 (1H, t, J = 3.5 Hz), 3.63 (3H, s), 2.89 (1H, dd, J = 4.0, 13.7 Hz), 1.22 (6H, s), 1.13, 1.12, 0.94, 0.91, 0.80 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 201.3, 178.4, 144.4, 143.9, 128.4, 122.0, 54.1, 51.8, 47.0, 45.9, 44.1, 43.3, 42.2, 41.6, 40.2, 38.7, 34.1, 33.3, 32.7, 32.5, 30.9, 27.8, 27.4, 26.1, 23.8, 23.6, 23.2, 22.0, 19.8, 18.9, 17.5. EIMS (70 eV) *m/z*: 482 $[M]^+$ (26), 446 (68), 422 (25), 262 (35), 203 (100). HREIMS: Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3387.

Methyl 2-Hydroxymethylene-3-oxoolean-12-en-28-oate (25).[19] To a stirred mixture of **B-3** (1084 mg, 2.31 mmol) and ethyl formate (97%) (707 mg, 9.26 mmol) in benzene (12 mL) was added NaOMe (501 mg, 9.27 mmol). The mixture was stirred at room temperature for 1 h. After the mixture was washed with 5% aqueous HCl solution twice, it was worked up according to the standard method to give **25** as an amorphous solid (1095 mg, 95%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (7:1)] and subsequent recrystallization from MeOH as colorless needles: mp 199–201 °C. UV (EtOH) λ_{max} (log ϵ): 296 (3.94) nm. IR (KBr): 3426, 2943, 2862, 1725, 1637, 1588 cm^{-1}. ^1H NMR ($CDCl_3$): δ 14.92 (1H, d, J = 3.1 Hz), 8.58 (1H, d, J = 3.1 Hz), 5.35 (1H, t, J = 3.7 Hz), 3.64 (3H, s), 2.90 (1H, dd, J = 4.2, 13.6 Hz), 2.29 (1H, d, J = 14.4 Hz), 1.92 (1H, d, J = 14.4 Hz), 1.20, 1.16, 1.12, 0.94 (each 3H, s), 0.91 (6H, s), 0.80 (3H, s). ^{13}C NMR ($CDCl_3$): δ 190.9, 188.6, 178.4, 144.0, 122.3, 106.0, 52.3, 51.8, 47.0, 46.0, 45.9, 42.0, 41.6, 40.3, 39.4, 39.3, 36.5, 34.1, 33.3, 32.5, 32.1, 30.9, 28.6, 27.9, 25.9, 23.8, 23.6, 23.3, 21.1, 19.7, 16.8, 14.7. EIMS (70 eV) *m/z*: 496 $[M]^+$ (4.4), 437 (23), 262 (38), 233 (20), 203 (100). HREIMS: Calcd for $C_{32}H_{48}O_4$: 496.3553. Found: 496.3550.

Methyl Isoxazolo[4,5-*b*]olean-12-en-28-oate (26). A mixture of **25** (994 mg, 2.0 mmol), hydroxylamine hydrochloride (1391 mg, 20 mmol) in water (1.8 mL) and EtOH (48 mL) was heated under reflux for 1 h. After EtOH was removed in vacuo, EtOAc was added to the resultant mixture. The EtOAc layer was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate gave a solid (1086 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (6:1) followed by hexanes–EtOAc (5:1)] to give **26** as an amorphous solid (934 mg, 86%): UV (EtOH) λ_{max} (log ϵ): 228 (3.65) nm. IR (KBr): 2940, 2864, 1725 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.98 (1H, s), 5.34 (1H, t, J = 3.5 Hz), 3.63 (3H, s), 2.89 (1H, dd, J = 4.4, 13.7 Hz), 2.42 (1H, d, J = 15.1 Hz), 1.30, 1.21, 1.15, 0.93, 0.90, 0.88, 0.79 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 178.4, 173.2, 150.4, 144.0, 122.3, 109.0, 53.7, 51.7, 46.9, 46.3, 46.0, 42.0, 41.6, 39.5, 38.9, 35.5, 34.9, 34.0, 33.3, 32.5, 32.1, 30.9, 29.0, 27.9, 25.9, 23.8, 23.5, 23.2, 21.6, 19.0, 16.7, 15.4. EIMS (70 eV) *m/z*: 493 $[M]^+$ (11), 434 (18), 262 (28), 249 (16), 203 (100). HREIMS: Calcd for $C_{32}H_{47}O_3N$: 493.3556. Found: 493.3556.

Methyl 2-Cyano-3-oxooolean-12-en-28-oate (27). To a stirred solution of **26** (887 mg, 1.80 mmol) in Et_2O (50 mL) and MeOH (25 mL) was added NaOMe (3.2 g) in an ice bath. The mixture was stirred at room temperature for 1 h. The mixture was diluted with a mixture of CH_2Cl_2 and Et_2O (1:2) (50 mL). After the extract was washed with 5% aqueous HCl solution, it was worked up according to the standard method to afford **27** as an amorphous solid (879 mg, 99%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (5:1)] as an amorphous solid: UV (EtOH) λ_{max} (log ϵ): 238 (3.88) nm. IR (KBr): 2946, 2870, 2202, 1724, 1633 cm^{-1}. ^1H NMR of major tautomer **27a** ($CDCl_3$): δ 6.15 (1H, brs), 5.31 (1H, t, J = 3.6 Hz), 3.63 (3H, s), 2.88 (1H, dd, J = 4.0, 13.6 Hz), 2.09 (1H, d, J = 15.0 Hz), 1.16, 1.13, 1.07, 0.95, 0.93, 0.90, 0.76 (each 3H, s). EIMS (70 eV) *m/z*: 493 $[M]^+$ (6.3), 434 (17), 262 (19), 249 (20), 203 (100). HREIMS: Calcd for $C_{32}H_{47}O_3N$: 493.3556. Found: 493.3548.

Methyl 2-Hydroxymethylene-3-oxours-12-en-28-oate (28).[23] **28** was prepared from **B-4** according to the same method as for **25** to give an amorphous solid (quantitative). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (7:1)] and subsequent recrystallization from MeOH as colorless needles: mp 170–171 °C. UV (EtOH) λ_{max} (log ϵ): 294 (3.86) nm. IR (KBr): 3426, 2947, 2921, 2866, 1727, 1637, 1590 cm^{-1}. ^1H NMR ($CDCl_3$): δ 14.91 (1H, brs), 8.57 (1H, s), 5.31 (1H, t, J = 3.7 Hz), 3.62 (3H, s), 2.31 (1H, d, J = 14.4 Hz), 2.27 (1H, d, J = 12.5 Hz), 1.95 (1H, d, J = 14.4 Hz), 1.19, 1.12, 1.10 (each 3H, s), 0.96 (3H, d, J = 6.0 Hz), 0.92 (3H, s), 0.87 (3H, d, J = 6.6 Hz), 0.81 (3H, s). ^{13}C NMR ($CDCl_3$): δ 191.0, 188.5, 178.2, 138.4, 125.6, 106.0, 53.2, 52.3, 51.7, 48.4, 45.7, 42.4, 40.3, 39.7, 39.5, 39.3, 39.1, 36.8, 36.4, 32.4, 30.9, 28.7, 28.2, 24.4, 23.7, 23.6, 21.4, 21.1, 19.7, 17.2, 17.0, 14.8. EIMS (70 eV) *m/z*: 496 $[M]^+$ (11), 437 (15), 262 (80), 233 (41), 203 (100). HREIMS: Calcd for $C_{32}H_{48}O_4$: 496.3553. Found: 496.3547.

Methyl Isoxazolo[4,5-*b*]urs-12-en-28-oate (29). **29** was prepared from **28** according to the same method as for **26** to give an amorphous solid (84%): UV (EtOH) λ_{max} (log ϵ): 228 (3.70) nm. IR (KBr): 2969, 2922, 2870, 1725 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.98 (1H, s), 5.31 (1H, t, J = 3.4 Hz), 3.62 (3H, s), 2.46 (1H, d, J = 15.0 Hz), 2.27 (1H, d, J = 11.1 Hz), 1.31, 1.22,

1.10 (each 3H, s), 0.96 (3H, d, $J = 6.3$ Hz), 0.90 (3H, s), 0.88 (3H, d, $J = 6.3$ Hz), 0.81 (3H, s). ^{13}C NMR ($CDCl_3$): δ 178.2, 173.2, 150.4, 138.4, 125.5, 109.1, 53.7, 53.2, 51.7, 48.3, 46.3, 42.3, 39.7, 39.3, 39.1, 38.8, 36.8, 35.8, 34.9, 32.4, 30.9, 29.1, 28.3, 24.4, 23.7, 23.5, 21.6, 21.4, 19.0, 17.2, 16.9, 15.6. EIMS (70 eV) *m/z*: 493 $[M]^+$ (9.1), 434 (20), 262 (65), 249 (33), 203 (100). HREIMS: Calcd for $C_{32}H_{47}O_3N$: 493.3556. Found: 493.3547.

Methyl 2-Cyano-3-oxours-12-en-28-oate (30). **30** was prepared from **29** according to the same method as for **27** to give an amorphous solid (quantitative). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (5:1)] as an amorphous solid: UV (EtOH) λ_{max} (log ϵ): 238 (3.93) nm. IR (KBr): 2947, 2870, 2203, 1724, 1631 cm^{-1}. ^{1}H NMR of major tautomer **30a** ($CDCl_3$): δ 5.92 (1H, brs), 5.28 (1H, t, $J = 3.5$ Hz), 3.61 (3H, s), 2.26 (1H, d, $J = 11.0$ Hz), 2.13 (1H, d, $J = 15.0$ Hz), 1.16, 1.13, 1.08, 1.07, 0.96 (each 3H, s), 0.95, 0.77 (each 3H, d, $J = 6.3$ Hz). EIMS (70 eV) *m/z*: 493 $[M]^+$ (6.8), 434 (19), 262 (62), 249 (44), 203 (100). HREIMS: Calcd for $C_{32}H_{47}O_3N$: 493.3556. Found: 493.3558.

Methyl 3-Hydroxy-2-methoxycarbonyloleana-2,12-dien-28-oate (31). A mixture of **B-3** (2.0 g, 4.27 mmol) and 1.8 M DMF solution of methoxymagnesium methyl carbonate (Stiles' reagent) (20 mL, 36 mmol) was heated under reflux for 2 h while a slow stream of N_2 was bubbled through the mixture with a pipet. To the mixture were added 5% aqueous HCl solution and EtOAc. The aqueous layer was extracted with EtOAc (three times). The combined organic layers were washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a solid (2.26 g). To a solution of the solid in THF (30 mL) was added excessive amount of ethereal diazomethane. The mixture was kept at room temperature for 10 min. The mixture was evaporated in vacuo to give a solid (2.38 g). The solid was subjected to flash column chromatography [hexanes–EtOAc (7:1)] to give **B-3** (330 mg) and **31** as crystals (1.66 g; 74%, 89% based on recovered **B-3**): mp 160–162 °C. UV (EtOH) λ_{max} (log ϵ): 262 (4.01) nm. IR (KBr): 2948, 2858, 1737, 1660, 1615 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 12.51 (1H, s), 5.33 (1H, t, $J = 3.7$ Hz), 3.74, 3.63 (each 3H, s), 2.89 (1H, dd, $J = 4.2$, 13.9 Hz), 2.35 (1H, d, $J = 15.7$ Hz), 1.18, 1.14, 1.10, 0.94 (each 3H, s), 0.91 (6H, s), 0.78 (3H, s). ^{13}C NMR ($CDCl_3$): δ 178.5, 177.9, 174.2, 143.8, 122.6, 94.3, 52.5, 51.8, 51.7, 47.0, 46.13, 46.09, 42.0, 41.7, 39.4, 38.6, 38.4, 35.7, 34.1, 33.3, 32.6, 32.1, 31.0, 28.8, 27.9, 26.0, 23.8, 23.6, 23.3, 20.4, 19.8, 16.8, 15.1. EIMS (70 eV) *m/z*: 526 $[M]^+$ (0.6), 494 (5.6), 479 (2.5), 466 (1.6), 435 (13), 262 (28), 203 (100). HREIMS: Calcd for $C_{33}H_{50}O_5$: 526.3658. Found: 526.3658.

2-Hydroxymethylene-3-oxoolean-12-en-28-oic Acid (32). To a stirred mixture of oleanonic acid (**B-1**)[10] (540 mg, 1.19 mmol) and ethyl formate (97%) (357 mg, 4.66 mmol) in THF (12 mL) was added NaOMe (258 mg, 4.78 mmol). The mixture was stirred at room temperature overnight. The mixture was acidified with 10% aqueous HCl solution. The mixture was extracted with EtOAc three times. The extract was worked up according to the standard method to give a solid (600 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1) followed by hexanes–EtOAc (4:1)] to afford **B-1** (168 mg) and **32** as a crystalline solid (260 mg; 45%, 66% based on recovered **B-1**): mp 200–203 °C dec. UV (EtOH) λ_{max} (log ϵ): 292 (3.93) nm. IR (KBr): 2946, 2654, 1732, 1694, 1644, 1587 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 14.91 (1H, brs), 8.59 (1H, s), 5.34 (1H, t, $J = 3.5$ Hz), 2.86 (1H, dd, $J = 4.5$, 13.9 Hz), 2.29 (1H, d, $J = 14.6$ Hz), 1.93 (1H, d, $J = 14.6$ Hz), 1.19, 1.16, 1.10, 0.94, 0.92, 0.91, 0.82 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 190.7, 188.8, 184.7, 143.8, 122.6, 105.9, 52.2, 46.8, 46.0, 45.9, 41.9, 41.2, 40.2, 39.34, 39.30, 36.5, 34.0, 33.3, 32.6, 32.0, 30.9, 28.6, 27.8, 25.9, 23.7, 23.5, 23.1, 21.0, 19.6, 17.0, 14.6. EIMS (70 eV) *m/z*: 482 $[M]^+$ (1.8), 438 (2.7), 436 (3.6), 248 (77), 203 (100). HREIMS: Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3392.

Evaluation Methods. 1. Reagents. Recombinant mouse IFN-γ (LPS content, <10 pg/mL) was purchased from Genzyme (Cambridge, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Inhibitory test compounds were dissolved in DMSO before addition to cell cultures; final concentrations of DMSO were 0.1% or less. Controls with DMSO alone were run in all cases.

2. Cell Culture. To obtain primary macrophages, female CD-1 mice, 5–10 weeks of age (Charles River Breeding Laboratories, Wilmington, MA), were injected intraperitoneally with 2 mL of 4% thioglycollate broth (Difco Laboratories, Detroit, MI). Four days after injection, peritoneal macrophages were harvested and processed according to Nathan's procedure.[7b] Cells were seeded in 96-well plates at 2×10^5 cells/well and incubated for 48 h with 20 ng/mL IFN-γ in the presence or absence of inhibitory test compounds.

3. Measurement of NO Production in Mouse Macrophages. Nitrite accumulation was used as an indicator of NO production in the medium and was assayed by the Griess reaction.[7a] Griess reagent (100 μL) was added to 100 μL of each supernatant from IFN-γ or inhibitory test compound-treated cells in triplicate. The protein determination was performed by Bradford protein assay. The plates were read at 550 nm against a standard curve of sodium nitrite.

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Synthetic Oleanane and Ursane Triterpenoids with Modified Rings A and C: A Series of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages

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Synthetic Oleanane and Ursane Triterpenoids with Modified Rings A and C: A Series of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages†

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We have designed and synthesized 16 new olean- and urs-1-en-3-one triterpenoids with various modified rings C as potential antiinflammatory and cancer chemopreventive agents and evaluated their inhibitory activities against production of nitric oxide induced by interferon-γ in mouse macrophages. This investigation revealed that 9(11)-en-12-one and 12-en-11-one functionalities in ring C increase the potency by about 2−10 times compared with the original 12-ene. Subsequently, we have designed and synthesized novel olean- and urs-1-en-3-one derivatives with nitrile and carboxyl groups at C-2 in ring A and with 9(11)-en-12-one and 12-en-11-one functionalities in ring C. Among them, we have found that methyl 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate (**25**), 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**26**), and methyl 2-carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate (**29**) have extremely high potency (IC_{50} = 0.1 nM level). Their potency is similar to that of dexamethasone although they do not act through the glucocorticoid receptor. Overall, the combination of modified rings A and C increases the potency by about 10 000 times compared with the lead compound, 3-oxooleana-1,12-dien-28-oic acid (**8**) (IC_{50} = 1 μM level). The selected oleanane triterpenoid, CDDO (**26**), was found to be a potent, multifunctional agent in various in vitro assays and to show antiinflammatory activity against thioglycollate−interferon-γ-induced mouse peritonitis.

Introduction

Oleanane and ursane triterpenoids are pentacyclic compounds with 30 carbon atoms, biosynthetically derived from the cyclization of squalene.[1] This is a vast class of natural products whose structural diversity includes a wide array of functional groups.[2] Many compounds of this group are reported to have various interesting biological, pharmacological, or medicinal activities including antiinflammatory and anticarcinogenic activities.[3] However, in many cases, the potency of these triterpenoids is relatively weak. Therefore, anticipating highly potent novel structures, we began bioassay-directed systematic drug design and synthesis of derivatives of commercially available oleanolic acid (**1**) and ursolic acid (**2**) (cf. Scheme 1).

To discover antiinflammatory and cancer chemopreventive drugs from these derivatives, we have adopted an assay system that measures inhibition of nitric oxide (NO) production induced by interferon-γ (IFN-γ) in mouse macrophages[4] as a preliminary screening assay system. In a previous paper,[5] we reported that olean-12-ene triterpenoids with a 1-en-3-one functionality having nitrile, methoxycarbonyl, and carboxyl groups at C-2 in ring A, **3**−**7**, show significant potency [IC_{50} = 0.01−0.1 μM level, about 10−100 times more potent than the lead compound **8** (IC_{50} = 1 μM level)] in this assay. As a continuation of this work, we have synthesized 16 new olean- and urs-1-en-3-one derivatives with various modified rings C, **9**−**24**, and evaluated their inhibitory activities in the above assay. This investigation revealed that 9(11)-en-12-one, 12-en-11-one, and 13-(18)-en-11-one functionalities in ring C increase the potency by about 2−10 times compared with the original 12-ene. Subsequently, we have designed and synthesized novel olean- and urs-1-en-3-one derivatives with nitrile, methoxycarbonyl, and carboxyl groups at C-2 in ring A and with 9(11)-en-12-one, 12-en-11-one, and 13-(18)-en-11-one functionalities in ring C, **25**−**35**. Among them, we have found that methyl 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate (**25**), 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**26**), and methyl 2-carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate (**29**) have extremely high potency (IC_{50} = 0.1 nM level). We report here the synthesis, inhibitory activity, and

† Part of this work has been reported in preliminary form: (a) Honda, T.; Finlay, H. J.; Gribble, G. W.; Suh, N.; Sporn, M. B. New enone derivatives of oleanolic acid and ursolic acid as inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1623−1628. (b) Honda, T.; Rounds, B. V.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. Design and synthesis of 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid, a novel and highly active inhibitor of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2711−2714. (c) Honda, T.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. Novel synthetic oleanane triterpenoids: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3429−3434.

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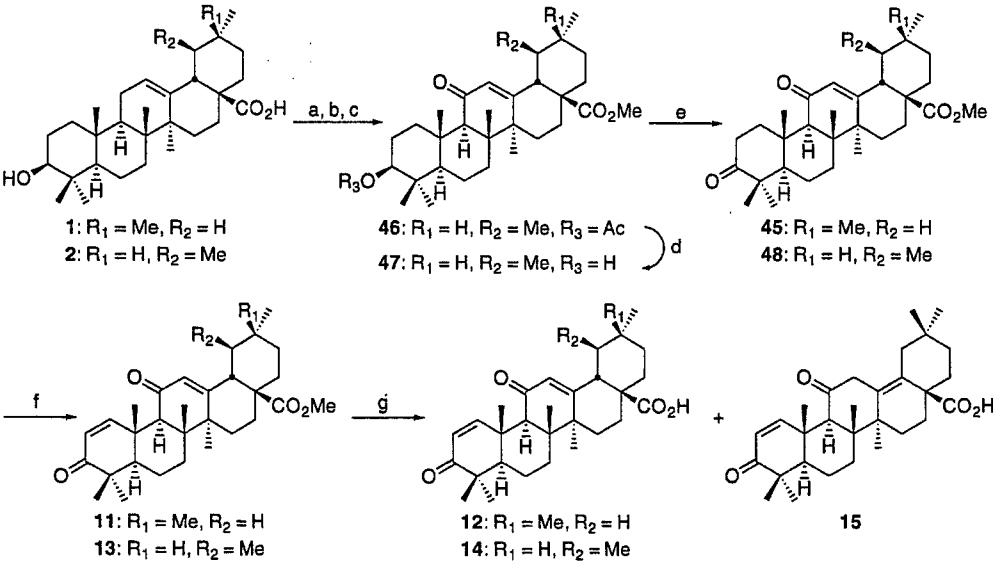
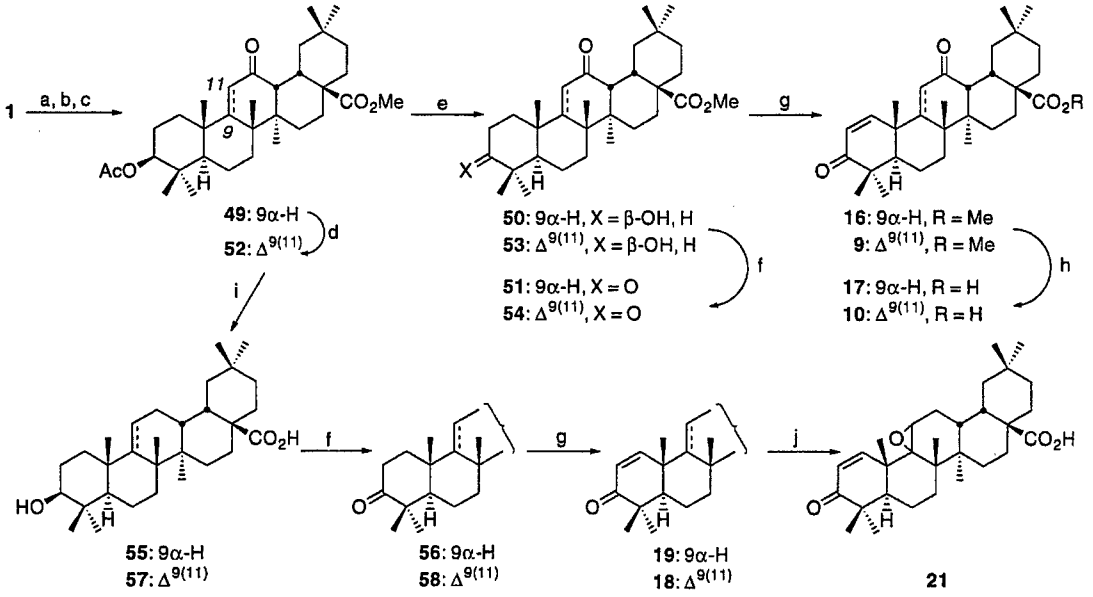
Scheme 1[a]



[a] Reagents: (a) CH_2N_2, Et_2O, THF; (b) Ac_2O, pyr; (c) CrO_3, pyr, CH_2Cl_2; (d) KOH, aq MeOH; (e) Jones; (f) PhSeCl, EtOAc, 30% H_2O_2, THF; (g) LiI, DMF.

Scheme 2[a]



[a] Reagents: (a) CH_2N_2, Et_2O, THF; (b) Ac_2O, pyr; (c) 30% H_2O_2, AcOH; (d) Br_2, HBr, AcOH; (e) KOH, aq MeOH; (f) Jones; (g) PhSeCl, EtOAc, 30% H_2O_2, THF; (h) LiI, DMF; (i) NH_2NH_2, KOH, diethylene glycol; (j) *m*CPBA, CH_2Cl_2.

structure–activity relationships (SARs) of these novel triterpenoids in detail.

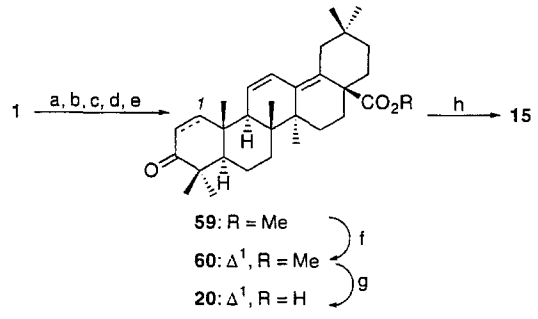
Chemistry

Modification of Ring C and Carboxyl Group at C-17. Enones **9**–**21** were designed and synthesized to discover what structures of ring C enhance potency in comparison with the original 12-ene, i.e., the lead compound **8**[5] (Schemes 1–3).[6] In addition, enones **22**–**24** were designed and synthesized to learn which functionality at C-17 is most appropriate (Scheme 4).

Enone **11** was prepared by introduction of a double bond at C-1 of known C-3 ketone **45**,[7] which was prepared in five steps from oleanolic acid (**1**), with phenylselenenyl chloride in ethyl acetate and sequential addition of 30% hydrogen peroxide ($PhSeCl–H_2O_2$) (yield, 97%).[8] Halogenolysis of **11** with lithium iodide (LiI) in *N*,*N*-dimethylformamide (DMF)[9] gave α,β- and β,γ-unsaturated ketones **12** and **15** in 43% and 22% yield, respectively. C-3 alcohol **47** was obtained quantitatively by alkaline hydrolysis (reflux) of known acetate **46**,[10] which was prepared in three steps from ursolic acid (**2**). Jones oxidation of **47** gave C-3 ketone **48** in 89% yield. Enone **13** was prepared in 93% yield from **48** by the same method as for **11**. Halogenolysis of **13** gave acid **14** in 58% yield.[11]

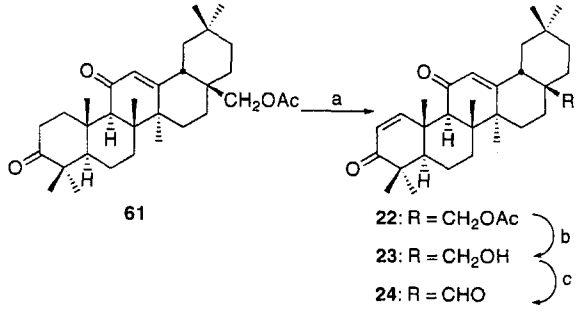
Similarly, enone **16** was synthesized in 74% yield via **50** and **51** from C-12 ketone **49**, which was prepared in three steps from **1** according to a known method,[12,13] and enone **9** was also synthesized in 60% yield via **53** and **54** from known C-12 ketone **52** which was prepared from **49** with bromine and hydrobromic acid in acetic acid.[14] Halogenolysis of enones **16** and **9** gave acids **17** and **10** in 62% and 68% yield, respectively. Enone **19**

Scheme 3[a]



[a] Reagents: (a) CH_2N_2, Et_2O, THF; (b) Ac_2O, pyr; (c) SeO_2, AcOH; (d) KOH, aq MeOH; (e) CrO_3, pyr, CH_2Cl_2; (f) PhSeCl, EtOAc, 30% H_2O_2, THF; (g) LiI, DMF; (h) Jones.

Scheme 4[a]



[a] Reagents: (a) PhSeCl, EtOAc, 30% H_2O_2, THF; (b) KOH, aq MeOH; (c) CrO_3, pyr, CH_2Cl_2.

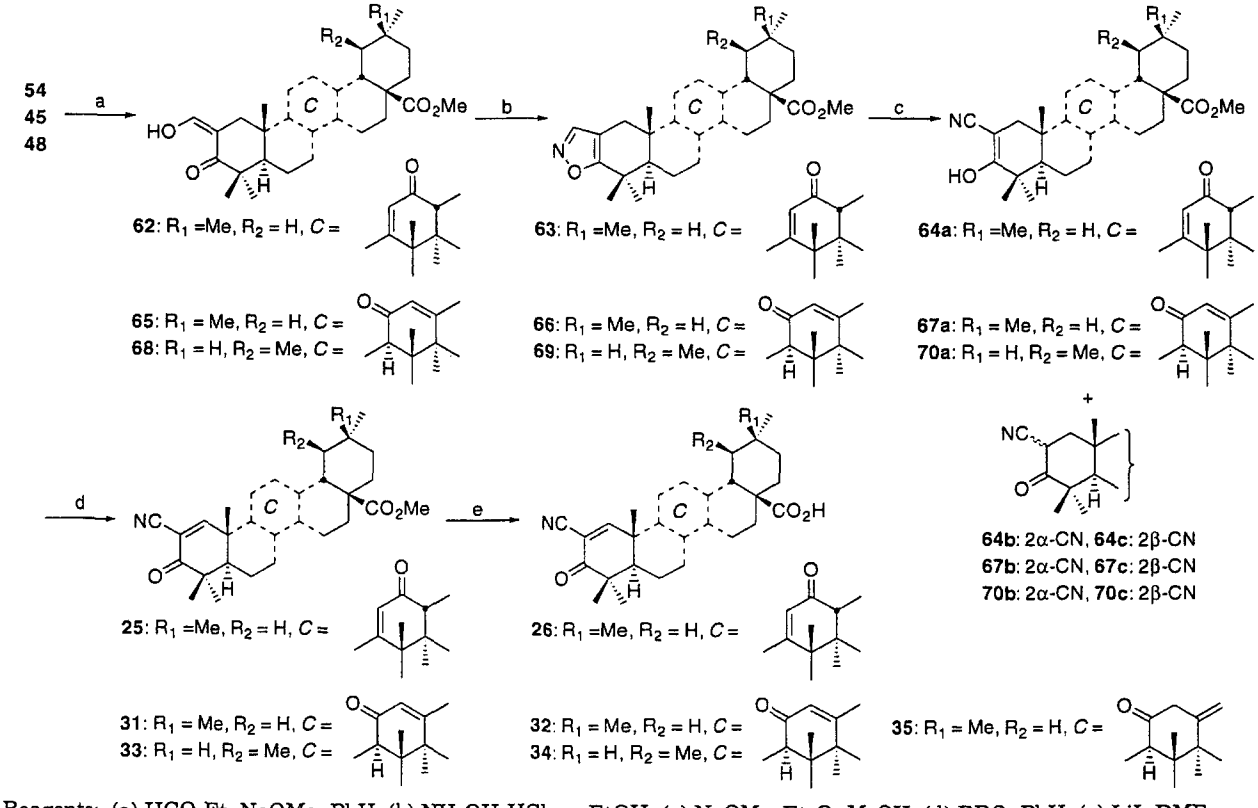
was obtained in 68% yield from known C-3 ketone **56**[15] which was synthesized via **55** from **49**. Enone **18** was obtained in 76% yield via **58** from acid **57**, which was prepared in 53% yield from **52** by Wolff–Kishner reduction. Epoxide **21**[16] was prepared in 46% yield by oxidation of **18** with *m*-chloroperbenzoic acid (*m*CPBA) in methylene chloride (CH_2Cl_2). Enone **20** was synthesized in 37% yield via **60** from known diene **59**[17] which was prepared in five steps from **1**. Interestingly, Jones oxidation of **20** afforded the same deconjugated enone **15** (yield, 28%) as halogenolysis of **11**. Enone **22** was prepared in 83% yield from krukovine A acetate (**61**), which was previously synthesized in our laboratory.[18] Alkaline hydrolysis (at room temperature)[19] of **22** gave enone **23** in 78% yield. Ratcliffe oxidation[20] of **23** with chromium trioxide and pyridine in CH_2Cl_2 afforded aldehyde **24** in 89% yield.

Among these new synthetic enones, **9**–**12** and **15** showed more inhibitory activity than the lead compound **8** on production of NO-induced IFN-γ in mouse macrophages (see Table 1). Overall, 9(11)-en-12-one, 12-en-11-one, and 13(18)-en-11-one functionalities in ring C increase the potency by about 2–10 times compared with the original 12-ene.

Combination of Modified Ring A with Ring C. On the basis of our previous results,[5] in which olean-12-ene triterpenoids with a 1-en-3-one functionality having nitrile, methoxycarbonyl, and carboxyl groups at C-2 in ring A, **3**–**7**, are about 10–100 times more potent than **8** (see Table 1), and the above results, we have designed and synthesized novel oleanane and ursane triterpenoids with modified rings A and C, **25**–**35**. In addition, to further discern SARs, amide **36** and enal **37** were designed and synthesized because amide **41** and enal **42** showed low potency and toxicity, respectively, in our previous evaluation (see Table 1).[5] The syntheses of these newly designed triterpenoids are illustrated in Schemes 5–7.

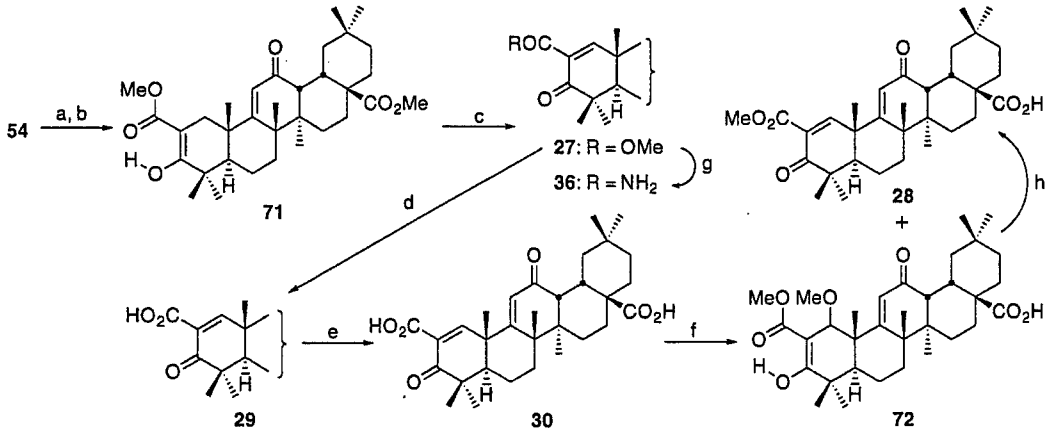
Hydroxymethylene **62**[21] was prepared in 99% yield by formylation of **54** with ethyl formate in the presence

Scheme 5[a]



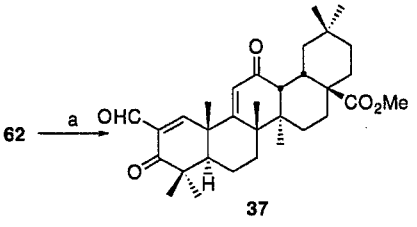
[a] Reagents: (a) HCO_2Et, NaOMe, PhH; (b) NH_2OH·HCl, aq EtOH; (c) NaOMe, Et_2O, MeOH; (d) DDQ, PhH; (e) LiI, DMF.

Scheme 6[a]



[a] Reagents: (a) Stiles' reagent, DMF; (b) CH_2N_2, Et_2O, THF; (c) PhSeCl, pyr, CH_2Cl_2, 30% H_2O_2, CH_2Cl_2; (d) KOH, aq MeOH; (e) LiI, DMF; (f) H_2SO_4, MeOH; (g) NH_3, MeOH; (h) SiO_2.

Scheme 7[a]



[a] Reagents: (a) PhSeCl, pyr, CH_2Cl_2, 30% H_2O_2, CH_2Cl_2.

of sodium methoxide in benzene.[22] Isoxazole **63** was obtained in 66% yield from **62** by the addition of hydroxylamine.[23] Cleavage of the isoxazole moiety of **63** with sodium methoxide gave nitrile **64** quantitatively.[23,24] CDDO methyl ester (**25**) was prepared in 92% yield by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **64** in benzene, although PhSeCl–H_2O_2 gave **25** in only 40% yield. Halogenolysis of **25** gave CDDO (**26**) in 68% yield. Similarly, olean-12-en-11-one derivative **31** was synthesized in 53% yield via **65**,[21] **66**, and **67**[24] from **45**. Halogenolysis of **31** gave α,β- and β,γ-unsaturated ketones **32** and **35** in 37% and 16% yield, respectively. Urs-12-en-11-one derivative **33** was also synthesized in 61% yield via **68**,[21] **69**, and **70**[24] from **48**. Halogenolysis of **33** gave acid **34** in 60% yield.[11]

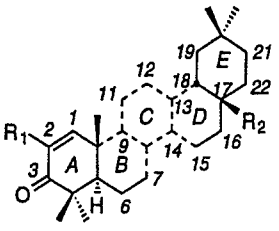
Ester **71** was prepared in 78% yield from C-3 ketone **54** by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,[25] followed by methylation with diazomethane. ^{1}H NMR showed that **71** in $CDCl_3$ is the single tautomer depicted in Scheme 6. Enone **27** was prepared from **71** by PhSeCl–pyridine in CH_2Cl_2 and sequential addition of 30% H_2O_2[26] (yield, 71%; 88% based on recovered **71**). Hydrolysis (reflux) of **27** with potassium hydroxide in aqueous methanol (MeOH) gave C-2 carboxylic acid **29** and decarboxylated enone **9** in 78% and 8% yield, respectively. Because of the steric hindrance of the methoxycarbonyl group at C-17 of **27**, the above conditions gave monoesters **29** and **9** selectively. Halogenolysis of **29** gave dicarboxylic acid **30** and decarboxylated enone **10** in 47% and 24% yield, respectively. Interestingly, methylation of **30** with MeOH under acidic conditions gave a mixture of desired monoester **28** and Michael adduct **72**.[27] The ratio of **28** to **72** was determined to be 4:5 by ^{1}H NMR. Because the adduct **72** was readily transformed into **28** under purification conditions (see Experimental Section), **28** was finally obtained in 82% yield from **30**. Amide **36** was prepared selectively from **27** with saturated ammonia–MeOH (yield, 49%; 88% based on recovered **27**). Enal **37** was synthesized from **62** according to the same method as for **27** (yield, 62%; 74% based on recovered **62**).

Biological Results and Discussion

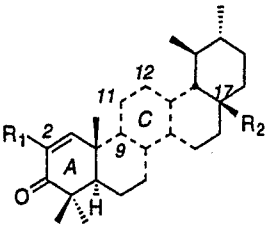
The inhibitory activities [IC_{50} (μM) value] of synthetic triterpenoids **3**–**44**, oleanolic acid (**1**), ursolic acid (**2**), hydrocortisone, and dexamethasone (both glucocoriticoids are used as positive controls) on NO production induced by IFN-γ in mouse macrophages are shown in Table 1. These derivatives are arranged categorically in order of the amplification of potency due to the structure of ring C. Among novel synthetic oleanane and ursane triterpenoids, **25**, CDDO (**26**), and **29** showed extremely high potency (IC_{50} = 0.1 nM level). Their potency is equivalent to that of dexamethasone although their inhibitory activity is not blocked by the glucocorticoid antagonist, RU-486,[28] which reverses the action of dexamethasone (data not shown).

This series of synthetic triterpenoids showed the following interesting SARs: (1) A 9(11)-en-12-one functionality is the strongest enhancer of potency among structures of ring C. Oleanane triterpenoids **10** and **9** (IC_{50} = 0.1 μM level) are about 10–100 times more potent than the lead compounds **8** (IC_{50} = 1 μM level) and **43** (IC_{50} = 10 μM level), respectively. (2) 12-En-11-one, 13(18)-en-11-one, and 12-one functionalities also enhance potency. Oleanane triterpenoids **11**, **12**, **15**, and **17** are more potent than **8**. Also, ursane triterpenoids **13** and **14** are more potent than **44**. (3) A 9(11)-ene functionality shows similar potency to the original 12-ene (compare **18** with **8**). (4) The saturated ring C, 11,-13(18)-diene, and 9,11-epoxide are less potent than the original 12-ene (compare **19**–**21** with **8**). (5) Carboxyl, methoxycarbonyl, and nitrile groups at C-2 enhance potency.[5] Oleanane triterpenoids **3**–**7** (IC_{50} = 0.01–0.1 μM level) are about 10–100 times more potent than **8**. Ursane triterpenoids **38** and **39** are more potent than **44**. (6) The combination of a 9(11)-en-12-one functionality with nitrile and carboxyl groups at C-2 enhances the potency synergistically. Oleanane triterpenoids **25**, CDDO (**26**), and **29** (IC_{50} = 0.1 nM level) are about 10 000 times more potent than **8** (see Figure 1). (7) Although compounds **27** and **30** were also expected to show similar

Table 1. Activity of Olean-1-en-3-one and Urs-1-en-3-one Triterpenoids



olean-1-en-3-one



urs-1-en-3-one

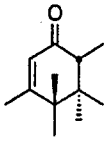
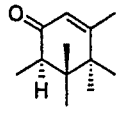
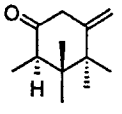
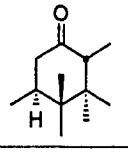
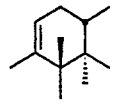
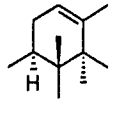
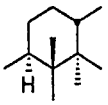
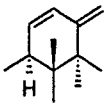
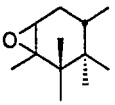
compd	skeleton[a]	structure of ring C	R_1 at C-2	R_2 at C-17	formula	analyses[b]	activity[c] IC_{50} (μM)
9	O		H	CO_2Me	$C_{31}H_{44}O_4 \cdot 1/3H_2O$	C,H	0.7
10	O		H	CO_2H	$C_{30}H_{42}O_4 \cdot 1/3H_2O$	C,H	0.2
25	O		CN	CO_2Me	$C_{32}H_{43}O_4N$	C,H,N	0.0001
26	O		CN	CO_2H	$C_{31}H_{41}O_4N$	C,H,N	0.0002
27	O		CO_2Me	CO_2Me	$C_{33}H_{46}O_6$	C,H	toxic[d]
28	O		CO_2Me	CO_2H	$C_{32}H_{44}O_6 \cdot 1/3H_2O$	C,H	0.1
29	O		CO_2H	CO_2Me	$C_{32}H_{44}O_6 \cdot 1/2H_2O$	C,H	0.0008
30	O		CO_2H	CO_2H	$C_{31}H_{42}O_6 \cdot 1/2H_2O$	C,H	0.2
36	O		$CONH_2$	CO_2Me	$C_{32}H_{45}O_5N \cdot 1/3H_2O$	C,H,N	0.1
37	O		CHO	CO_2Me	$C_{32}H_{44}O_5 \cdot 5/4H_2O$	C,H	0.1
11	O		H	CO_2Me	$C_{31}H_{44}O_4$	C,H	2.8
12	O		H	CO_2H	$C_{30}H_{42}O_4 \cdot 1/4H_2O$	C,H	1.1
13	U		H	CO_2Me	$C_{31}H_{44}O_4 \cdot 1/4H_2O$	C,H	8.9
14	U		H	CO_2H	$C_{30}H_{42}O_4 \cdot 1/4H_2O$	C,H	5.1
22	O		H	CH_2OAc	$C_{32}H_{46}O_4$	C,H	>40
23	O		H	CH_2OH	$C_{30}H_{44}O_3 \cdot 1/2H_2O$	C,H	3.0
24	O		H	CHO	$C_{30}H_{42}O_3 \cdot 1/2H_2O$	C,H	3.8
31	O		CN	CO_2Me	$C_{32}H_{43}O_4N \cdot 1/3H_2O$	C,H,N	0.02
32	O		CN	CO_2H	$C_{31}H_{41}O_4N \cdot 1/3H_2O$	C,H,N	0.04
33	U		CN	CO_2Me	$C_{32}H_{43}O_4N$	C,H,N	0.1
34	U		CN	CO_2H	$C_{31}H_{41}O_4N \cdot H_2O$	C,H,N	0.8
15	O		H	CO_2H	$C_{30}H_{42}O_4 \cdot 3/4H_2O$	C,H	2.6
35	O		CN	CO_2H	$C_{31}H_{41}O_4N \cdot 1/2H_2O$	C,H,N	0.07
16	O		H	CO_2Me	$C_{31}H_{46}O_4$	C,H	14
17	O		H	CO_2H	$C_{30}H_{44}O_4 \cdot 2/3H_2O$	C,H	3.3
18	O		H	CO_2H	$C_{30}H_{44}O_3 \cdot 1/2H_2O$	C,H	5.2
43	O		H	CO_2Me	$C_{31}H_{46}O_3$	ref 32	31
8	O		H	CO_2H	$C_{30}H_{44}O_3 \cdot 3/4H_2O$	ref 5	5.6
44	U		H	CO_2H	$C_{30}H_{44}O_3$	ref 33	13
3	O		CN	CO_2Me	$C_{32}H_{45}O_3N \cdot 1/4H_2O$	ref 5	0.7
4	O		CN	CO_2H	$C_{31}H_{43}O_3N \cdot 1/2H_2O$	ref 5	0.6
38	U		CN	CO_2Me	$C_{32}H_{45}O_3N \cdot 3/4H_2O$	ref 5	5.1
39	U		CN	CO_2H	$C_{31}H_{43}O_3N \cdot H_2O$	ref 5	6.2
5	O		CO_2Me	CO_2Me	$C_{33}H_{48}O_5$	ref 5	0.9
40	O		CO_2Me	CO_2H	$C_{32}H_{46}O_5$	ref 5	2.2
6	O		CO_2H	CO_2Me	$C_{32}H_{46}O_5 \cdot 1/2H_2O$	ref 5	0.8
7	O		CO_2H	CO_2H	$C_{31}H_{44}O_5$	ref 5	0.07
41	O		$CONH_2$	CO_2Me	$C_{32}H_{47}O_4N \cdot 3/4H_2O$	ref 5	14
42	O		CHO	CO_2Me	$C_{32}H_{46}O_4$	ref 5	toxic[d]

Table 1 (Continued)

compd	skeleton[a]	structure of ring C	R_1 at C-2	R_2 at C-17	formula	analyses[b]	activity[c] IC_{50} (μM)
19	O		H	CO_2H	$C_{30}H_{46}O_3 \cdot 2/3H_2O$	C,H	8.5
20	O		H	CO_2H	$C_{30}H_{42}O_3 \cdot H_2O$	C,H	9.7
21	O		H	CO_2H	$C_{30}H_{44}O_4 \cdot 1/2H_2O$	C,H	36
1	oleanolic acid						>40
2	ursolic acid						toxic[e]
	hydrocortisone						0.01
	dexamethasone						0.0001

[a] O, olean-1-en-3-one; U, urs-1-en-3-one. [b] C, H, and N analyses were within ±0.4% of the theoretical values. [c] Details of the evaluation method are described in the Experimental Section. IC_{50} values of compounds **7**, **25**, **26**, **29**, **31**, **32**, **35**, hydrocortisone, and dexamethasone were determined in the range of 0.1 pM−1 μM (10-fold dilutions). The other compounds were assayed in the range of 0.01−40 μM (4-fold dilutions). Values are an average of two separate experiments. [d] Compounds **27** and **42** were toxic to cells above 1 μM and were not active below 1 μM. [e] Ursolic acid (**2**) was toxic to cells above 10 μM and was not active below 10 μM.

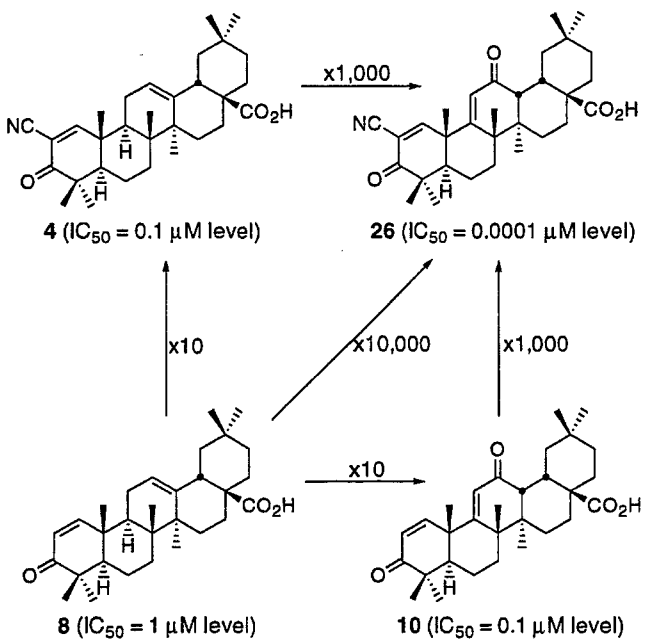


Figure 1. SARs between CDDO (**26**) and its lead compounds **4**, **8**, and **10**.

high potency to CDDO from the perspective of SARs, they did not (compare them with **5** and **7**). The reason diacid **30** did not show high potency might be that the higher polarity than that of monoacids **26** and **29** influences the bioavailability and permeability toward macrophages. (8) The combination of a 9(11)-en-12-one functionality with amide and formyl groups at C-2 does not enhance potency as strongly as a nitrile or carboxyl group as expected from the consideration of the activity of oleana-1,12-dien-3-one with amide and formyl groups at C-2 (compare **36** and **37** with **41** and **42**, respectively). (9) The combination of 12-en-11-one and 13(18)-en-11-one functionalities with a nitrile group at C-2 also strongly enhances the potency. Oleanane triterpenoids **31**, **32**, and **35** (IC_{50} = 0.01 μM level) are about 100 times more potent than **8**. Also, ursane triterpenoids **33** and **34** (IC_{50} = 0.1 μM level) are about 100 times more potent than **44** (IC_{50} = 10 μM level). (10) The oleanane skeleton is more potent than the ursane skeleton. Oleanane derivatives **3**, **4**, **8**, **11**, **12**, **31**, and **32** are more potent than ursane derivatives **38**, **39**, **44**, **13**, **14**, **33**, and **34**, respectively. (11) Acetoxymethyl, hydroxymethyl, and formyl groups at C-17 decrease potency compared with the carboxyl group at C-17 (compare **22**−**24** with **12**). (12) The role of methoxycarbonyl and carboxyl groups at C-17 is ambiguous. In some analogues, the carboxyl group is more potent than the methoxycarbonyl group: acids **7**, **8**, **17**, and **28** are more potent than esters **6**, **43**, **16**, and **27**, respectively. For other analogues, the carboxyl and methoxycarbonyl groups show similar potency: acids **4**, **26**, **32**, and **39** show similar potency to esters **3**, **25**, **31**, and **38**, respectively. Acids and esters with a nitrile group at C-2 seem to show this tendency although the reason is unknown. Lastly, acids **30** and **40** are less potent than esters **29** and **5**, respectively.

The selected oleanane triterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**26**), was found to be a potent, multifunctional agent in various in vitro assays.[29] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts.[30] CDDO inhibits proliferation of many human tumor cell lines. CDDO blocks de novo synthesis of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. CDDO will protect rat brain hippocampal neurons from cell death induced by β-amyloid. The above potencies have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture. In addition, CDDO shows antiinflammatory activity against thioglycollate−IFN-γ-induced mouse peritonitis (0.1 μmol of CDDO/mouse, ip: a complete

suppression of both NO production and iNOS protein synthesis; 0.01 μmol of CDDO/mouse, ip: more than 50% suppression in these measurements).[31] CDDO may be a potential drug candidate for inflammatory diseases and chemoprevention of cancer.

Currently, further biological evaluation of CDDO, **25**, and **29** in vitro and in vivo for both antiinflammation and chemoprevention is in progress. Further studies on the mechanism of action of these compounds also are in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. UV and IR spectra were recorded on a Hewlett-Packard 8451A UV/VIS spectrophotometer and a Perkin-Elmer 600 series FTIR spectrophotometer, respectively. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 Fourier transform spectrometer unless otherwise stated. The chemical shifts are reported in δ (ppm) using the δ 7.27 signal of $CHCl_3$ (^{1}H NMR) and δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as an internal standard unless otherwise stated. Low-resolution mass spectra and high-resolution MS data were obtained on a Micromass 70-VSE unless otherwise stated. Elemental microanalysis was performed by Atlantic Microlab Inc. TLC and preparative TLC (prep-TLC) were performed with Merck precoated TLC plates silica gel 60 F_{254}. Flash column chromatography was done with Select Scientific silica gel (230–400 mesh). The standard workup method was as follows: an organic extract was washed with saturated aqueous $NaHCO_3$ solution (three times) followed by saturated aqueous NaCl solution (three times), then dried over anhydrous $MgSO_4$, and filtered. The filtrate was evaporated in vacuo.

Methyl 3,12-Dioxooleana-1,9(11)-dien-28-oate (9). A solution of **54** (145 mg, 0.30 mmol) and phenylselenenyl chloride (98%) (69 mg, 0.35 mmol) in EtOAc (7 mL) was stirred at room temperature for 2.5 h. To the stirred mixture was added water (1.5 mL). After most of the aqueous layer was removed, THF (2.7 mL) and 30% H_2O_2 (0.24 mL) were added to the organic layer. The mixture was stirred at room temperature for 1 h. The mixture was worked up according to the standard method to give a crude solid (134 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (5:1)] to give **9** as an amorphous solid (96 mg, 67%): $[\alpha]^{23}_D$ +58° (*c* 0.64, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.20) nm. IR (KBr): 2948, 2872, 1723, 1666, 1598 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.33 (1H, d, J = 10.5 Hz), 6.00 (1H, s), 5.92 (1H, d, J = 10.5 Hz), 3.69 (3H, s), 3.04 (1H, ddd, J = 3.4, 4.6, 13.4 Hz), 2.91 (1H, d, J = 4.6 Hz), 1.41, 1.31, 1.19, 1.12, 1.01, 1.00, 0.89 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 203.7, 199.8, 178.4, 171.6, 155.0, 126.1, 123.8, 52.1, 49.8, 48.5, 47.4, 45.8, 44.9, 42.2, 42.0, 36.0, 34.7, 33.5, 33.0, 32.3, 31.7, 30.8, 28.2, 27.3, 27.1, 24.7, 23.3, 22.8, 21.84, 21.81, 18.6. EIMS (70 eV) *m/z*: 480 [M]$^+$ (99), 465 (100), 446 (42), 405 (27), 315 (41), 244 (44). HREIMS Calcd for $C_{31}H_{44}O_4$: 480.3240. Found: 480.3238. Anal. (Table 1).

3,12-Dioxooleana-1,9(11)-dien-28-oic Acid (10). A mixture of **9** (82 mg, 0.17 mmol) and LiI (405 mg) in dry DMF (2 mL) was heated under reflux for 7.5 h. To the mixture were added water and 5% aqueous HCl solution. The mixture was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) (three times). The extract was worked up according to the standard method to give an amorphous solid (78 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (1:1)] to give **10** as a crystalline solid (54 mg, 68%). An analytically pure sample was obtained by recrystallization from a mixture of hexanes and EtOAc (2:1) as colorless needles: mp >270 °C dec; $[\alpha]^{23}_D$ +63° (*c* 0.42, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.14) nm. IR (KBr): 3117, 2973, 2941, 2867, 1734, 1710, 1671, 1639, 1598 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.33 (1H, d, J = 10.6 Hz), 6.02 (1H, s), 5.93 (1H, d, J = 10.6 Hz), 3.02 (1H, ddd, J = 3.4, 4.9, 13.7 Hz), 2.96 (1H, d, J = 4.9 Hz), 1.41, 1.32, 1.19, 1.11, 1.02, 1.00, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 203.8, 199.6, 183.9, 171.7, 155.0, 126.1, 123.8, 49.9, 48.4, 47.2, 45.8, 44.8, 42.2, 41.9, 35.9, 34.6, 33.4, 33.1, 32.3, 31.6, 30.8, 28.2, 27.3, 27.1, 24.8, 23.2, 22.7, 21.83, 21.75, 18.5. EIMS (70 eV) *m/z*: 466 [M]$^+$ (100), 451 (42), 301 (17), 244 (45). HREIMS Calcd for $C_{30}H_{42}O_4$: 466.3083. Found: 466.3064. Anal. (Table 1).

Methyl 3,11-Dioxooleana-1,12-dien-28-oate (11). 11 was prepared from methyl 3,11-dioxoolean-12-en-28-oate (**45**)[7] according to the same method as for **9** to give a crystalline solid (97%). This material was used for the next reaction without further purification. An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (3:1)], followed by recrystallization from a mixture of hexanes and EtOAc (3:1) as crystals: mp 189–191 °C; $[\alpha]^{24}_D$ +152° (*c* 0.34, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.26) nm. IR (KBr): 2942, 2861, 1725, 1666, 1648 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.79 (1H, d, J = 10.3 Hz), 5.81 (1H, d, J = 10.3 Hz), 5.74 (1H, s), 3.66 (3H, s), 3.05 (1H, dd, J = 4.6, 14.9 Hz), 2.67 (1H, s), 2.08 (1H, ddd, J = 4.0, 13.7, 13.7 Hz), 1.39 (6H, s), 1.16, 1.11, 0.97, 0.96, 0.95 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 204.7, 199.3, 177.6, 170.4, 161.8, 127.6, 124.8, 55.7, 52.9, 52.1, 46.3, 45.3, 44.9, 44.4, 43.9, 42.0, 39.1, 33.8, 33.0, 32.3, 31.7, 30.9, 28.0, 27.8, 23.8, 23.6, 23.0, 21.7, 20.1, 19.4, 18.3. EIMS (70 eV) *m/z*: 480 [M]$^+$ (88), 465 (15), 421 (24), 397 (52), 276 (36), 257 (47), 217 (100). HREIMS Calcd for $C_{31}H_{44}O_4$: 480.3240. Found: 480.3231. Anal. (Table 1).

3,11-Dioxooleana-1,12-dien-28-oic Acid (12) and 3,11-Dioxooleana-1,13(18)-dien-28-oic Acid (15). 12 and **15** were prepared from **11** by the similar method as for **10** except that the reaction time was 2 h. The reaction mixture was subjected to prep-TLC [hexanes–EtOAc (3:5)] to give **12** as an amorphous solid (43%) and **15** as a crystalline solid (22%). **12**: $[\alpha]^{24}_D$ +161° (*c* 0.51, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.35) nm. IR (KBr): 3154, 2948, 2869, 1732, 1652, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.77 (1H, d, J = 10.3 Hz), 5.81 (1H, d, J = 10.3 Hz), 5.74 (1H, s), 3.02 (1H, dd, J = 4.3, 13.6 Hz), 2.67 (1H, s), 2.09 (1H, ddd, J = 5.2, 14.3, 14.3 Hz), 1.39, 1.38, 1.15, 1.08, 0.97, 0.96, 0.95 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 204.8, 199.4, 183.2, 170.1, 161.8, 127.9, 124.9, 55.7, 52.9, 46.2, 45.4, 44.9, 44.3, 44.0, 41.8, 39.1, 33.8, 33.0, 32.4, 31.7, 30.9, 28.0, 27.9, 23.8, 23.6, 22.7, 21.7, 20.2, 19.7, 18.2. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 467 [M + H]$^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{42}O_4$ + H: 467.3161. Found: 467.3161. Anal. (Table 1). **15**: mp >190 °C dec; $[\alpha]^{25}_D$ −16° (*c* 0.26, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 210 (4.16), 226 (4.15), 300 (3.16) nm. IR (KBr): 3200, 2946, 2866, 1692 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, d, J = 10.1 Hz), 5.82 (1H, d, J = 10.1 Hz), 3.56 (1H, d, J = 17.8 Hz), 2.88 (1H, d, J = 17.8 Hz), 2.65 (1H, s), 2.31 (2H, m), 2.09 (1H, m), 1.44, 1.31, 1.16, 1.10, 0.96, 0.93, 0.76 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 208.6, 204.8, 181.9, 160.8, 133.8, 129.9, 125.1, 58.0, 52.9, 48.1, 44.8, 44.3, 44.1, 43.4, 41.1, 39.1, 36.7, 35.7, 33.03, 32.95, 32,8, 32.2, 27.7, 26.6, 24.2, 21.8, 20.2, 20.1, 19.2, 18.8. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 467 [M + H]$^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{42}O_4$ + H: 467.3161. Found: 467.3187. Anal. (Table 1).

Methyl 3,11-Dioxoursa-1,12-dien-28-oate (13). 13 was prepared from **48** according to the same method as for **9** to give a crystalline solid (93%). An analytically pure sample was obtained by recrystallization from a mixture of hexanes and EtOAc (3:1) as crystals: mp 172–174 °C; $[\alpha]^{24}_D$ +150° (*c* 0.49, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.26) nm. IR (KBr): 2973, 2948, 2866, 1726, 1670, 1655, 1610 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.75 (1H, d, J = 10.3 Hz), 5.82 (1H, d, J = 10.3 Hz), 5.71 (1H, s), 3.63 (3H, s), 2.64 (1H, s), 2.47 (1H, d, J = 11.7 Hz), 2.11 (1H, ddd, J = 4.6, 14.7, 14.7 Hz), 1.41, 1.33, 1.16, 1.11 (each 3H, s), 0.98 (3H, d, J = 7.2 Hz), 0.97 (3H, s), 0.89 (3H, d, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$): δ 204.7, 198.8, 177.3, 164.5, 161.8, 130.4, 124.8, 55.5, 53.1, 53.0, 52.1, 47.9, 45.0, 44.9, 44.2, 39.0, 38.82, 38.79, 36.1, 32.5, 30.5, 28.7, 27.8, 24.0, 21.8, 21.3, 21.2, 20.1, 19.4, 18.3, 17.3. EIMS (70 eV) *m/z*: 480 [M]$^+$ (84), 465

(19), 421 (15), 397 (100), 257 (38), 217 (39). HREIMS Calcd for $C_{31}H_{44}O_4$: 480.3240. Found: 480.3239. Anal. (Table 1).

3,11-Dioxoursa-1,12-dien-28-oic Acid (14). **14** was prepared from **13** by the similar method as for **10** except that the reaction time was 1.25 h. The reaction mixture was crystallized from a mixture of hexanes and EtOAc (2:1) to give **14** as crystals (58%). An analytically pure sample was obtained by recrystallization from MeOH as colorless needles: mp >275 °C dec; $[\alpha]^{24}_D$ +157° (*c* 0.29, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 247 (4.17) nm. IR (KBr): 3116, 2983, 2950, 2930, 1720, 1668, 1628 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.74 (1H, d, J = 10.3 Hz), 5.82 (1H, d, J = 10.3 Hz), 5.71 (1H, s), 2.65 (1H, s), 2.44 (1H, d, J = 11.2 Hz), 2.13 (1H, m), 1.41, 1.34, 1.15, 1.08 (each 3H, s), 0.99 (3H, d, J = 7.2 Hz), 0.97 (3H, s), 0.89 (3H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 204.8, 199.0, 183.1, 164.3, 161.7, 130.6, 124.8, 55.4, 52.9, 52.8, 47.7, 45.0, 44.9, 44.2, 39.0, 38.8, 38.7, 36.2, 32.5, 30.4, 28.6, 27.8, 23.7, 21.7, 21.3, 21.1, 20.2, 19.6, 18.2, 17.2. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 467 $[M + H]^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{42}O_4$ + H: 467.3161. Found: 467.3202. Anal. (Table 1).

3,11-Dioxooleana-1,13(18)-dien-28-oic Acid (15). To a solution of **20** (106 mg, 0.24 mmol) in acetone (6.5 mL) was added Jones reagent (0.36 mL) dropwise in an ice bath. The mixture was stirred at room temperature for 30 min. After removal of acetone, water was added to the resultant mixture. The aqueous mixture was extracted with CH_2Cl_2 (three times). The extract was worked up according to the standard method to give a solid (80 mg). The solid was subjected to prep-TLC [hexanes–EtOAc (1.2:1.0)] to give **15** as a crystalline solid (31 mg, 28%).

Methyl 3,12-Dioxoolean-1-en-28-oate (16). **16** was prepared from **51** according to the same method as for **9**. The crude solid was subjected to flash column chromatography [hexanes–EtOAc (3:1) followed by hexanes–EtOAc (2:1)] to give **16** as an amorphous solid (75%): $[\alpha]^{24}_D$ +2.1° (*c* 0.39, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 234 (3.85) nm. IR (KBr): 2946, 2867, 1724, 1700, 1671 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.95 (1H, d, J = 10.3 Hz), 5.84 (1H, d, J = 10.3 Hz), 3.70 (3H, s), 2.82 (1H, ddd, J = 3.5, 4.2, 13.4 Hz), 2.68 (1H, d, J = 4.2 Hz), 2.49 (1H, dd, J = 4.6, 16.4 Hz), 2.33 (1H, dd, J = 13.3, 16.4 Hz), 1.16, 1.11, 1.10, 1.06, 0.99, 0.97, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 210.4, 204.8, 178.5, 157.2, 126.0, 53.4, 52.2, 52.1, 47.5, 44.8, 44.2, 42.4, 42.3, 39.5, 38.6, 36.4, 34.6, 33.5, 33.0, 32.2, 31.6, 30.8, 27.82, 27.76, 23.3, 22.9, 21.6, 20.8, 19.1, 18.5, 16.6. EIMS (70 eV) *m/z*: 482 $[M]^+$ (5.5), 467 (42), 407 (100), 278 (25), 218 (64). HREIMS Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3387. Anal. (Table 1).

3,12-Dioxoolean-1-en-28-oic Acid (17). **17** was prepared from **16** by the similar method as for **10** except that the reaction time was 4.5 h. The crude material was subjected to prep-TLC [hexanes–EtOAc (1:2)] to give **17** as a crystalline solid (62%): mp 243–245 °C dec; $[\alpha]^{24}_D$ +2.3° (*c* 0.27, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 234 (3.89) nm. IR (KBr): 3166, 2946, 2866, 1722, 1696, 1668, 1651 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.96 (1H, d, J = 10.4 Hz), 5.84 (1H, d, J = 10.4 Hz), 2.79 (2H, m), 2.51 (1H, dd, J = 4.9, 15.9 Hz), 2.35 (1H, dd, J = 13.2, 15.9 Hz), 1.17, 1.11 (each 3H, s), 1.10 (6H, s), 1.00, 0.98, 0.93 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 210.2, 204.9, 184.2, 157.2, 126.0, 53.3, 52.2, 47.4, 44.8, 44.1, 42.4, 42.3, 39.5, 38.6, 36.2, 34.6, 33.5, 33.2, 32.0, 31.6, 30.8, 27.8, 23.3, 22.8, 21.6, 20.7, 19.1, 18.5, 16.7. EIMS (70 eV) *m/z*: 468 $[M]^+$ (9.7), 453 (15), 407 (39), 218 (19), 83 (100). HREIMS Calcd for $C_{30}H_{44}O_4$: 468.3240. Found: 468.3221. Anal. (Table 1).

3-Oxooleana-1,9(11)-dien-28-oic Acid (18). **18** was prepared from **58** according to the same method as for **9**. The crude solid was subjected to prep-TLC [hexanes–EtOAc (2:1)] to give **18** as a crystalline solid (80%). An analytically pure sample was obtained by recrystallization from MeOH as colorless needles: mp >240 °C dec; $[\alpha]^{24}_D$ +55° (*c* 0.28, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 234 (3.93) nm. IR (KBr): 3138, 3053, 2959, 2930, 2869, 1727, 1693, 1645 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.42 (1H, d, J = 10.4 Hz), 5.85 (1H, d, J = 10.4 Hz), 5.63 (1H, t, J = 3.4 Hz), 1.35, (3H, s), 1.16 (6H, s), 1.07, 0.94 (each 3H, s), 0.90 (6H, s). ^{13}C NMR ($CDCl_3$): δ 205.0, 185.0, 157.9, 147.7, 124.4, 118.6, 49.9, 48.1, 44.7, 44.1, 41.3, 38.6, 36.2, 35.6, 34.4, 33.7, 33.6, 33.2, 31.8, 30.8, 28.5, 28.0, 27.2, 26.9, 26.3, 23.6, 23.4, 21.7, 18.8, 18.7. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 453 $[M + H]^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{44}O_3$ + H: 453.3369. Found: 453.3390. Anal. (Table 1).

3-Oxoolean-1-en-28-oic Acid (19). **19** was prepared from 3-oxoolean-28-oic acid (**56**)[15] according to the same method as for **9**. The crude solid was subjected to flash column chromatography [hexanes–EtOAc (3:1)] to give **19** as an amorphous solid (68%): $[\alpha]^{24}_D$ +30° (*c* 0.55, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 236 (3.90) nm. IR (KBr): 3200, 2944, 2866, 1729, 1692, 1672 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.11 (1H, d, J = 10.2 Hz), 5.82 (1H, d, J = 10.2 Hz), 2.22 (1H, m), 1.13, 1.06, 1.04, 0.99, 0.96, 0.92, 0.88 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.7, 184.9, 159.8, 125.4, 53.5, 48.1, 44.8, 44.7, 42.9, 40.8, 39.7, 37.4, 36.7, 36.5, 34.5, 33.6, 33.4, 32.5, 30.6, 28.5, 28.0, 26.9, 23.6, 23.3, 21.6, 19.2, 17.2, 16.9. FABMS (NBA, by a Micromass ZAB-SE) *m/z*: 455 $[M + H]^+$. HRFABMS (by a Micromass 70-SE-4F) Calcd for $C_{30}H_{46}O_3$ + H: 455.3525. Found: 455.3518. Anal. (Table 1).

3-Oxooleana-1,11,13(18)-trien-28-oic Acid (20). **20** was prepared from **60** by the similar method as for **10** except that the reaction time was 4 h. The crude solid was subjected to prep-TLC [hexanes–EtOAc (2.5:1)] to give **20** as an amorphous solid (56%): $[\alpha]^{24}_D$ −88° (*c* 0.44, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 246 (4.35), 252 (4.35) nm. IR (KBr): 3167, 3036, 2944, 2863, 1727, 1695, 1672 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.27 (1H, d, J = 10.1 Hz), 6.57 (1H, dd, J = 2.9, 10.5 Hz), 5.89 (1H, d, J = 10.1 Hz), 5.81 (1H, dd, J = 1.5, 10.5 Hz), 2.57 (1H, d, J = 14.2 Hz), 2.29 (2H, m), 1.174, 1.170, 1.10, 1.00, 0.98, 0.86, 0.82 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.7, 182.8, 159.1, 136.6, 132.5, 126.5, 125.7, 125.4, 53.4, 48.4, 48.3, 45.0, 42.4, 41.6, 40.8, 39.3, 37.0, 35.6, 32.9, 32.7, 32.4, 31.9, 27.7, 25.1, 24.3, 21.32, 21.27, 20.0, 19.2, 16.9. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 451 $[M + H]^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{42}O_3$ + H: 451.3212. Found: 451.3240. Anal. (Table 1).

9,11-Epoxy-3-oxoolean-1-en-28-oic Acid (21). A mixture of **18** (57 mg, 0.13 mmol) and *m*CPBA (60%) (43 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature overnight. After the mixture was diluted with a mixture of CH_2Cl_2 and Et_2O (1:2), it was worked up according to the standard method to give a solid (65 mg). The solid was subjected to prep-TLC [hexanes–EtOAc (1.5:1)] to give **21** as a crystalline solid (27 mg, 46%). An analytically pure sample was obtained by recrystallization from MeOH as colorless needles: mp 253–254 °C; $[\alpha]^{24}_D$ −14° (*c* 0.25, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 236 (3.88) nm. IR (KBr): 2970, 2945, 1688 cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.55 (1H, d, J = 10.4 Hz), 5.85 (1H, d, J = 10.4 Hz), 3.02 (1H, s), 1.39 (3H, s), 1.07 (6H, s), 1.04, 0.96, 0.92, 0.87 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 204.5, 184.4, 154.4, 125.2, 67.8, 60.2, 47.9, 45.3, 44.9, 42.3, 41.5, 38.4, 37.3, 35.7, 34.3, 33.6, 33.3, 30.8, 30.0, 28.2, 27.9, 26.9, 24.9, 23.6, 23.3, 21.1, 20.6, 18.7, 18.6. FABMS (NBA, by a VG analytical ZAB 2SE) *m/z*: 469 $[M + H]^+$. HRFABMS (by a VG analytical ZAB 2SE) Calcd for $C_{30}H_{44}O_4$ + H: 469.3318. Found: 469.3314. Anal. (Table 1).

3,11-Dioxooleana-1,12-dien-28-yl Acetate (22). **22** was prepared from 3,11-dioxoolean-12-en-28-yl acetate (**61**)[18] according to the same method as for **9**. The crude solid was subjected to prep-TLC [hexanes–EtOAc (3:1)] to give **22** as an amorphous solid (83%): $[\alpha]^{24}_D$ +131° (*c* 0.45, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 246 (4.31) nm. IR (KBr): 2949, 2868, 1742, 1665 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.72 (1H, d, J = 10.1 Hz), 5.79 (1H, d, J = 10.1 Hz), 5.67 (1H, s), 3.97 (1H, d, J = 11.2 Hz), 3.71 (1H, d, J = 11.2 Hz), 2.66 (1H, s), 2.29 (1H, dd, J = 4.2, 13.2 Hz), 2.07 (3H, s), 2.03 (1H, ddd, J = 4.4, 13.9, 13.9 Hz), 1.404, 1.397, 1.18, 1.15, 1.11, 0.93, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 204.7, 198.9, 171.2, 170.2, 161.7, 128.3, 124.8, 70.3, 55.8, 53.0, 45.7, 44.99, 44.96, 43.8, 42.9, 39.0, 36.0, 33.9, 33.0, 32.1, 31.2, 31.0, 27.8, 26.1, 23.7, 23.5, 22.1, 21.7, 21.1, 20.2, 19.0, 18.3. EIMS (70 eV) *m/z*: 494 $[M]^+$ (100), 446 (92), 411

(41), 406 (37), 351 (19). HREIMS Calcd for $C_{32}H_{46}O_4$: 494.3396. Found: 494.3396. Anal. (Table 1).

28-Hydroxyoleana-1,12-diene-3,11-dione (23). A solution of **22** (47 mg, 0.095 mmol) and KOH (300 mg) in MeOH (3 mL) was stirred at room temperature for 20 min. The mixture was acidified with 5% aqueous HCl solution. The aqueous mixture was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) (three times). The extract was worked up according to the standard method to give an amorphous solid (42 mg). The solid was subjected to prep-TLC [hexanes–EtOAc (1.7:1)] to give **23** as an amorphous solid (34 mg, 78%): $[\alpha]^{24}_D$ +145° (*c* 0.50, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 250 (4.13) nm. IR (KBr): 3477, 2947, 2865, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.72 (1H, d, J = 10.3 Hz), 5.80 (1H, d, J = 10.3 Hz), 5.67 (1H, s), 3.48 (1H, d, J = 11.0 Hz), 3.25 (1H, d, J = 11.0 Hz), 2.67 (1H, s), 2.21 (1H, dd, J = 3.8, 13.6 Hz), 1.97 (1H, ddd, J = 4.4, 13.7, 13.7 Hz), 1.41, 1.40 (each 3H, s), 1.16 (6H, s), 1.11, 0.93, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 204.8, 199.1, 171.4, 161.8, 128.0, 124.8, 69.8, 55.7, 53.0, 45.8, 45.1, 45.0, 43.9, 43.0, 39.0, 37.2, 34.0, 33.1, 32.2, 31.3, 30.8, 27.8, 26.1, 23.6, 21.8, 21.7, 20.3, 19.0, 18.4. EIMS (70 eV) *m/z*: 452 $[M]^+$ (100), 437 (15), 434 (16), 383 (16), 364 (50), 248 (46). HREIMS Calcd for $C_{30}H_{44}O_3$: 452.3290. Found: 452.3292. Anal. (Table 1).

Oleana-1,12-diene-3,11,28-trione (24). To a stirred mixture of CrO_3 (70 mg, 0.70 mmol) and pyridine (110 mg, 1.39 mmol) in dry CH_2Cl_2 (2 mL) was added a solution of **23** (53 mg, 0.12 mmol) in dry CH_2Cl_2 (1.5 mL). The mixture was stirred at room temperature for 15 min. The mixture was worked up according to Ratcliffe's procedure[20] to give a crude solid of **24** (47 mg, 89%). The solid was recrystallized from a mixture of hexanes and EtOAc (2:1) to give **24** as colorless needles (31 mg, 59%): mp >267 °C dec; $[\alpha]^{24}_D$ +160° (*c* 0.27, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 248 (4.15) nm. IR (KBr): 2944, 2864, 1719, 1674, 1644 cm^{-1}. ^1H NMR ($CDCl_3$): δ 9.40 (1H, s), 7.76 (1H, d, J = 10.3 Hz), 5.80 (1H, d, J = 10.3 Hz), 5.77 (1H, s), 2.84 (1H, dd, J = 4.3, 13.6 Hz), 2.64 (1H, s), 2.10 (1H, ddd, J = 3.9, 14.3, 14.3 Hz), 1.38 (6H, s), 1.15, 1.10 (each 3H, s), 0.96 (6H, s), 0.93 (3H, s). ^{13}C NMR ($CDCl_3$): δ 205.4, 204.7, 199.0, 169.1, 161.7, 128.0, 124.8, 55.7, 53.0, 49.1, 45.4, 44.9, 44.3, 43.9, 40.1, 39.1, 33.2, 32.9, 32.4, 30.9, 27.9, 27.30, 27.26, 23.5, 23.3, 21.7, 21.6, 20.1, 19.6, 18.3. EIMS (70 eV) *m/z*: 450 $[M]^+$ (100), 446 (64), 367 (45), 362 (31), 246 (36). HREIMS Calcd for $C_{30}H_{42}O_3$: 450.3134. Found: 450.3129. Anal. (Table 1).

Methyl 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oate (25). A mixture of **64** (1.51 g, 2.97 mmol) and DDQ (98%) (0.77 g, 3.32 mmol) in dry benzene (80 mL) was heated under reflux for 30 min. After insoluble matter was removed by filtration, the filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [benzene–acetone (10:1)] to give **25** as an amorphous solid (1.38 g, 92%): $[\alpha]^{23}_D$ +33° (*c* 0.68, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 244 (4.07) nm. IR (KBr): 2950, 2872, 2233, 1722, 1690, 1665 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.04 (1H, s), 5.96 (1H, s), 3.68 (3H, s), 3.02 (1H, ddd, J = 3.4, 4.9, 13.4 Hz), 2.92 (1H, d, J = 4.9 Hz), 1.47, 1.31, 1.24, 1.15, 0.99, 0.98, 0.88 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.0, 196.8, 178.3, 168.6, 165.9, 124.2, 114.7, 114.6, 52.1, 49.8, 47.8, 47.3, 45.9, 45.2, 42.7, 42.2, 35.9, 34.6, 33.4, 32.9, 31.8, 31.6, 30.8, 28.1, 27.1, 26.8, 24.7, 23.2, 22.7, 21.8, 21.7, 18.4. EIMS (70 eV) *m/z*: 505 $[M]^+$ (100), 490 (81), 430 (42), 315 (47), 269 (40). HREIMS Calcd for $C_{32}H_{43}O_4N$: 505.3192. Found: 505.3187. Anal. (Table 1).

2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic Acid (26). A mixture of **25** (612 mg, 1.21 mmol) and LiI (3.0 g) in dry DMF (10 mL) was heated under reflux for 4 h. To the mixture were added water and 5% aqueous HCl solution. The mixture was extracted with EtOAc (three times). The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give an amorphous solid. The solid was subjected to flash column chromatography [hexanes–EtOAc (1:1) followed by CH_2Cl_2–MeOH (15:1)] to give crude **26** (530 mg). The crude product was purified by recrystallization from benzene to give crystals. To remove benzene completely, the crystals were dissolved in CH_2Cl_2 (20 mL) and the solvent was evaporated in vacuo to give benzene-free **26** as an amorphous solid (405 mg, 68%): $[\alpha]^{22}_D$ +33 ° (*c* 0.28, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.21) nm. IR (KBr): 2950, 2867, 2235, 1692, 1665 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.05 (1H, s), 6.00 (1H, s), 3.06–2.98 (2H, m), 1.48, 1.34, 1.25, 1.16, 1.02, 1.00, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.0, 196.8, 183.7, 168.8, 165.9, 124.2, 114.7, 114.5, 49.8, 47.8, 47.1, 45.9, 45.2, 42.7, 42.3, 35.8, 34.5, 33.3, 33.0, 31.8, 31.5, 30.8, 28.1, 27.1, 26.8, 24.8, 23.2, 22.6, 21.72, 21.71, 18.4. EIMS (70 eV) *m/z*: 491 $[M]^+$ (100), 476 (62), 445 (29), 430 (27), 269 (94). HREIMS Calcd for $C_{31}H_{41}O_4N$: 491.3036. Found: 491.3020. Anal. (Table 1).

Methyl 2-Methoxycarbonyl-3,12-dioxooleana-1,9(11)-dien-28-oate (27). To a solution of phenylselenenyl chloride (98%) (78 mg, 0.40 mmol) in CH_2Cl_2 (3.2 mL) in an ice bath was added a solution of pyridine (35 mg, 0.44 mmol) in CH_2Cl_2 (0.8 mL). After 15 min, a solution of **71** (108 mg, 0.20 mmol) in CH_2Cl_2 (1.4 mL) was added and the mixture was stirred an additional 1 h. After the mixture was washed with 10% aqueous HCl solution (1.6 mL) twice, 30% H_2O_2 (0.2 mL) was added to the stirred mixture in the ice bath. After an additional 40 min, the mixture was worked up according to the standard method to give a solid (108 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (2:1)] to afford **71** (21 mg) and **27** as colorless needles (76 mg; 71%, 88% based on recovered **71**): mp 187–188 °C; $[\alpha]^{23}_D$ +35° (*c* 0.38, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 246 (4.06) nm. IR (KBr): 2944, 2867, 1722, 1664, 1597 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.05 (1H, s), 6.09 (1H, s), 3.79, 3.69 (each 3H, s), 3.04 (1H, ddd, J = 3.5, 4.5, 13.9 Hz), 2.94 (1H, d, J = 4.5 Hz), 1.37, 1.30, 1.18, 1.17, 1.01, 0.99, 0.88 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.6, 199.4, 178.3, 170.8, 165.0, 160.7, 129.9, 125.2, 52.5, 52.1, 50.0, 48.3, 47.4, 46.0, 45.8, 42.3, 42.0, 36.0, 34.6, 33.4, 32.9, 31.7, 30.8, 28.2, 28.1, 27.3, 24.6, 23.3, 22.8, 21.7, 21.4, 18.8. EIMS (70 eV) *m/z*: 538 $[M]^+$ (20), 523 (40), 506 (100), 315 (47). HREIMS Calcd for $C_{33}H_{46}O_6$: 538.3294. Found: 538.3289. Anal. (Table 1).

2-Methoxycarbonyl-3,12-dioxooleana-1,9(11)-dien-28-oic Acid (28). A solution of **30** (33 mg, 0.064 mmol) in MeOH (3.1 mL) containing concentrated H_2SO_4 (0.09 mL) was heated under reflux for 25 min. After water was added to the mixture, it was extracted with EtOAc (three times). The extract was worked up according to the standard method to give a solid (31 mg). The solid was a mixture of **28** and 3-hydroxy-1-methoxy-2-methoxycarbonyl-12-oxooleana-2,9(11)-dien-28-oic acid (**72**). The solid was subjected to prep-TLC [hexanes–EtOAc (1:1)] to give only **28** as a crystalline solid (27 mg, 82%). An analytically pure sample was obtained by recrystallization from a mixture of hexanes and EtOAc (2:1) as colorless needles: mp >265 °C dec; $[\alpha]^{23}_D$ +34° (*c* 0.42, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 240 (4.09) nm. IR (KBr): 3118, 2977, 2940, 2869, 1718, 1692, 1636 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.06 (1H, s), 6.11 (1H, s), 3.81 (3H, s), 3.09–2.98 (2H, m), 1.38, 1.34, 1.20, 1.19, 1.04, 1.02, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.7, 199.2, 183.5, 170.9, 165.1, 160.7, 130.0, 125.3, 52.6, 50.0, 48.3, 47.2, 46.0, 45.9, 42.3, 42.0, 35.9, 34.6, 33.4, 33.1, 31.7, 31.6, 30.8, 28.2, 28.1, 27.3, 24.7, 23.2, 22.7, 21.7, 21.4, 18.8. EIMS (70 eV) *m/z*: 524 $[M]^+$ (17), 509 (24), 492 (100), 446 (38), 302 (31). HREIMS Calcd for $C_{32}H_{44}O_6$: 524.3138. Found: 524.3142. Anal. (Table 1). **72**:[34] ^1H NMR ($CDCl_3$): δ 13.06 (1H, s), 5.93 (1H, s), 4.46 (1H, s), 3.82 (3H, s), 3.21 (3H, s), 3.03 (2H, m), 2.12 (1H, dd, J = 3.8, 10.4 Hz), 1.26, 1.22, 1.13, 1.07, 1.05, 1.02, 0.92 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.2, 184.1, 182.1, 174.8, 174.0, 124.4, 96.9, 57.3, 51.9, 50.1, 47.4, 46.0, 45.7, 44.7, 42.8, 41.5, 39.5, 36.1, 34.7, 33.4, 33.2, 31.7, 31.2, 30.9, 28.5, 24.3, 23.8, 23.3, 23.2, 22.8, 21.2, 20.9, 18.5. EIMS (70 eV) *m/z*: 556 $[M]^+$ (3.0), 538 (54), 524 (61), 509 (35), 492 (96), 446 (86), 315 (100). HREIMS Calcd for $C_{33}H_{48}O_7$: 556.3400. Found: 556.3410.

Methyl 2-Carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate (29). A mixture of **27** (273 mg, 0.51 mmol) and KOH (1.6 g) in water (5.3 mL) and MeOH (16 mL) was heated under reflux for 15 min. After the mixture was acidified with 10% aqueous HCl solution, it was extracted with EtOAc (three

times). The extract was washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a solid (264 mg). The solid was recrystallized from MeOH to afford **29** as colorless needles (174 mg). The solid (75 mg) which was obtained from the mother liquid was subjected to flash column chromatography [hexanes−EtOAc (1:1)] to give **9** (19 mg, 8%) and **29** as colorless needles (33 mg, total 78%): mp 155−156 °C dec; $[\alpha]^{23}_D$ +50° (*c* 0.30, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 254 (4.14) nm. IR (KBr): 2950, 2872, 1756, 1722, 1664 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.77 (1H, s), 6.17 (1H, s), 3.70 (3H, s), 3.04 (1H, ddd, J = 3.6, 4.5, 13.2 Hz), 2.92 (1H, d, J = 4.5 Hz), 1.48, 1.34, 1.29, 1.22 (each 3H, s), 1.00 (6H, s), 0.90 (3H, s). ^{13}C NMR ($CDCl_3$): δ 207.6, 199.1, 178.4, 169.1, 168.5, 164.3, 124.5, 123.8, 52.1, 49.9, 47.7, 47.4, 45.9, 45.7, 42.5, 42.2, 35.9, 34.6, 33.4, 32.9, 31.8, 31.7, 30.8, 28.2, 27.5, 27.1, 24.8, 23.2, 22.8, 22.0, 21.8, 18.5. EIMS (70 eV) *m/z*: 524 $[M]^+$ (12), 509 (31), 506 (74), 480 (52), 465 (83), 405 (56), 315 (66), 175 (100). HREIMS Calcd for $C_{32}H_{44}O_6$: 524.3138. Found: 524.3138. Anal. (Table 1).

2-Carboxy-3,12-dioxooleana-1,9(11)-dien-28-oic Acid (30).[35] A mixture of **29** (120 mg, 0.23 mmol) and LiI (545 mg) in dry DMF (1.6 mL) was heated under reflux for 30 min. The reaction mixture was worked up according to the same method as for **26** to give a solid (125 mg). The solid was recrystallized from a mixture of hexanes and EtOAc (1:2) to afford **30** as colorless needles (36 mg). The solid which was obtained from the mother liquid was subjected to flash column chromatography [hexanes−EtOAc (1:2)] to give **10** (26 mg, 24%) and **30** as colorless needles (19 mg, total 47%): mp >260 °C dec; $[\alpha]^{24}_D$ +52° (*c* 0.28, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 256 (4.17) nm. IR (KBr): 3269, 2956, 2928, 1750, 1728, 1658, 1631, 1595 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.77 (1H, s), 6.18 (1H, s), 3.04 (1H, ddd, J = 3.5, 4.9, 13.6 Hz), 2.98 (1H, d, J = 4.9 Hz), 1.48, 1.36, 1.30, 1.23 (each 3H, s), 1.02 (6H, s), 0.91 (3H, s). EIMS (70 eV) *m/z*: 510 $[M]^+$ (12), 492 (100), 466 (71), 451 (75), 405 (48), 301 (37). HREIMS Calcd for $C_{31}H_{42}O_6$: 510.2981. Found: 510.2979. Anal. (Table 1).

Methyl 2-Cyano-3,11-dioxooleana-1,12-dien-28-oate (31). **31** was prepared from **67** according to the same method as for **25** to give an amorphous solid (80%): $[\alpha]^{24}_D$ +97° (*c* 0.49, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 250 (4.24) nm. IR (KBr): 2944, 2867, 2233, 1726, 1686, 1656, 1617 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.59 (1H, s), 5.77 (1H, s), 3.65 (3H, s), 3.06 (1H, dd, J = 4.0, 13.7 Hz), 2.69 (1H, s), 2.08 (1H, ddd, J = 4.1, 13.6, 13.6 Hz), 1.41, 1.38, 1.21, 1.15, 0.97, 0.96, 0.95 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 198.4, 197.8, 177.5, 173.0, 171.5, 127.3, 115.1, 113.5, 54.5, 52.2, 52.0, 46.3, 45.5, 45.3, 44.4, 44.1, 42.1, 40.0, 33.8, 33.0, 31.9, 31.6, 30.9, 28.0, 27.8, 23.8, 23.6, 23.0, 21.7, 19.6, 19.4, 18.2. EIMS (70 eV) *m/z*: 505 $[M]^+$ (100), 445 (22), 417 (27), 370 (20). HREIMS Calcd for $C_{32}H_{43}O_4N$: 505.3192. Found: 505.3200. Anal. (Table 1).

2-Cyano-3,11-dioxooleana-1,12-dien-28-oic Acid (32)[35] and 2-Cyano-3,11-dioxooleana-1,13(18)-dien-28-oic Acid (35). **32** and **35** were prepared from **31** by the similar method as for **26**. The reaction mixture was subjected to prep-TLC [hexanes−EtOAc−MeOH (50:100:1.5)] to give **32** as a crystalline solid (37%) and **35** as an amorphous solid (16%). **32**: mp >270 °C dec; $[\alpha]^{24}_D$ +101° (*c* 0.28, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 250 (4.23) nm. IR (KBr): 3228, 2944, 2867, 2233, 1732, 1689, 1656 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.58 (1H, s), 5.78 (1H, s), 3.04 (1H, dd, J = 3.7, 13.9 Hz), 2.69 (1H, s), 2.11 (1H, ddd, J = 3.9, 13.7, 13.7 Hz), 1.42, 1.40, 1.22, 1.14, 1.00, 0.973, 0.968 (each 3H, s). EIMS (70 eV) *m/z*: 491 $[M]^+$ (34), 445 (31), 397 (26), 257 (36), 189 (59), 95 (100). HREIMS Calcd for $C_{31}H_{41}O_4N$: 491.3036. Found: 491.3034. Anal. (Table 1). **35**: $[\alpha]^{25}_D$ −1.7° (*c* 0.47, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 210 (3.94), 240 (4.02), 304 (2.89) nm. IR (KBr): 3178, 2948, 2867, 2234, 1726, 1694, 1611 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.25 (1H, s), 3.60 (1H, d, J = 19.2 Hz), 2.91 (1H, d, J = 19.2 Hz), 2.68 (1H, s), 1.47, 1.30, 1.22, 1.15, 0.97, 0.94, 0.77 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 208.2, 197.7, 182.0, 171.8, 133.0, 130.5, 115.1, 113.9, 56.9, 52.0, 48.1, 45.2, 44.2, 44.0, 43.5, 41.1, 40.0, 36.7, 35.7, 33.1, 32.8, 32.4, 32.2, 27.6, 26.5, 24.2, 21.8, 20.1, 19.6, 19.3, 18.7. EIMS (70 eV) *m/z*: 491 $[M]^+$ (5.3), 461 (55), 445 (100), 351 (38), 310 (29), 257 (50). HREIMS Calcd for $C_{32}H_{41}O_4N$: 491.3036. Found: 491.3040. Anal. (Table 1).

Methyl 2-Cyano-3,11-dioxoursa-1,12-dien-28-oate (33). **33** was prepared from **70** according to the same method as for **25** to give a crystalline solid (90%): mp >275 °C dec; $[\alpha]^{25}_D$ +91° (*c* 0.36, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 250 (4.22) nm. IR (KBr): 2984, 2937, 2866, 2232, 1725, 1687, 1658, 1614 cm^{-1}. ^1H NMR (500 MHz, by a Varian Unityplus, $CDCl_3$): δ 8.55 (1H, s), 5.74 (1H, s), 3.63 (3H, s), 2.68 (1H, s), 2.49 (1H, d, J = 11.5 Hz), 2.12 (1H, m), 1.44, 1.34, 1.21, 1.15 (each 3H, s), 0.99 (3H, d, J = 6.4 Hz), 0.97 (3H, s), 0.89 (3H, d, J = 6.4 Hz). ^{13}C NMR (125.705 MHz, by a Varian Unityplus, $CDCl_3$): δ 197.9, 197.8, 177.2, 172.9, 165.6, 130.1, 115.1, 113.5, 54.2, 53.1, 52.1, 52.0, 47.8, 45.2, 45.1, 44.4, 39.9, 38.8, 36.0, 32.1, 30.4, 28.6, 27.8, 24.0, 21.7, 21.2, 21.1, 19.6, 19.4, 18.2, 17.3. EIMS (70 eV) *m/z*: 505 $[M]^+$ (62), 490 (15), 446 (19), 445 (19), 430 (23), 411 (47), 256 (37), 217 (37), 189 (69), 119 (100). HREIMS Calcd for $C_{32}H_{43}O_4N$: 505.3192. Found: 505.3200. Anal. (Table 1).

2-Cyano-3,11-dioxoursa-1,12-dien-28-oic Acid (34).[35] A mixture of **33** (155 mg, 0.31 mmol) and LiI (750 mg) in dry DMF (2.4 mL) was heated under reflux for 1.5 h. The reaction mixture was poured into water to give a solid. The solid was filtered and washed with water (several times). The crude solid (140 mg) was crystallized from a mixture of hexanes and EtOAc (2:1) to give **34** as crystals (90 mg, 60%). An analytically pure sample was obtained by crystallization from a mixture of CH_2Cl_2 and MeOH as crystals: mp >285 °C dec; $[\alpha]^{25}_D$ +119° (*c* 0.25, DMSO). UV (DMSO) λ_{max} (log ϵ): 264 (4.16) nm. IR (KBr): 3117, 3050, 2983, 2951, 2930, 2873, 2231, 1719, 1685, 1624 cm^{-1}. ^1H NMR [DMSO-d_6, internal standard: δ 2.50 (CD_2HSOCD_3)]: δ 8.49 (1H, s), 5.54 (1H, s), 2.95 (1H, s), 2.33 (1H, d, J = 11.2 Hz), 2.11 (1H, dd, J = 3.9, 13.2, 13.2 Hz), 1.35, 1.30, 1.13, 1.06 (each 3H, s), 0.942 (3H, d, J = 4.2 Hz), 0.935 (3H, s), 0.84 (3H, d, J = 6.4 Hz). EIMS (70 eV) *m/z*: 465 $[M - CN]^+$ (36), 446 $[M - CO_2H]^+$ (100), 420 (4.0), 405 (11), 315 (17), 244 (19). HREIMS Calcd for $C_{31}H_{41}O_4N - CN$: 465.3005. Found: 465.3010. Calcd for $C_{31}H_{41}O_4N - CO_2H$: 446.3059. Found: 446.3060. Anal. (Table 1).

Methyl 2-Aminocarbonyl-3,12-dioxooleana-1,9(11)-dien-28-oate (36). A solution of **27** (41.5 mg, 0.78 mmol) in saturated ammonia MeOH (4 mL) was kept at room temperature overnight. The mixture was evaporated in vacuo to give a residue (41 mg). The residue was subjected to flash column chromatography [hexanes−EtOAc (1:1.5)] to give **27** (18.5 mg) and **36** as an amorphous solid (19.6 mg; 49%, 88% based on recovered **27**): $[\alpha]^{24}_D$ +42° (*c* 0.36, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 242 (4.23) nm. IR (KBr): 3433, 3334, 2949, 2871, 1725, 1692, 1666 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.64 (1H, s), 8.35 (1H, d, J = 3.3 Hz), 6.22 (1H, s), 5.73 (1H, d, J = 3.3 Hz), 3.69 (3H, s), 3.05 (1H, ddd, J = 3.7, 4.5, 13.2 Hz), 2.92 (1H, d, J = 4.5 Hz), 1.41, 1.32 (each 3H, s), 1.20, 1.01 (each 6H, s), 0.90 (3H, s). ^{13}C NMR ($CDCl_3$): δ 204.4, 199.2, 178.5, 169.9, 165.3, 164.8, 127.8, 125.1, 52.1, 50.0, 47.8, 47.4, 46.2, 45.8, 42.2, 42.0, 35.9, 34.7, 33.4, 33.0, 31.73, 31.70, 30.8, 28.4, 28.2, 27.7, 24.7, 23.3, 22.9, 21.9, 21.8, 18.8. EIMS (70 eV) *m/z*: 523 $[M]^+$ (2.2), 508 (9.1), 506 (21), 446 (9.6), 315 (6.9), 84 (100). HREIMS Calcd for $C_{32}H_{45}O_5N$: 523.3298. Found: 523.3292. Anal. (Table 1).

Methyl 2-Formyl-3,12-dioxooleana-1,9(11)-dien-28-oate (37). **37** was prepared from **62** according to the same method as for **27** to give an amorphous solid (62%, 74% based on recovered **62**): $[\alpha]^{24}_D$ −3.7° (*c* 0.39, $CHCl_3$). UV (EtOH) λ_{max} (log ϵ): 254 (4.05) nm. IR (KBr): 2944, 2867, 1722, 1704, 1668, 1611 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.02 (1H, s), 8.11 (1H, s), 6.14 (1H, s), 3.70 (3H, s), 3.05 (1H, ddd, J = 3.7, 4.5, 13.2 Hz), 2.93 (1H, d, J = 4.5 Hz), 1.44, 1.33, 1.23, 1.19 (each 3H, s), 1.00 (6H, s), 0.89 (3H, s). ^{13}C NMR ($CDCl_3$): δ 202.2, 199.3, 189.8, 178.4, 169.9, 161.4, 131.5, 124.7, 52.1, 49.9, 48.2, 47.4, 46.0, 45.4, 42.3, 42.1, 36.0, 34.7, 33.4, 33.0, 31.9, 31.7, 30.8, 28.2, 27.5, 27.2, 24.7, 23.3, 22.8, 21.8, 21.6, 18.7. EIMS (70 eV) *m/z*: 508 $[M]^+$ (37), 493 (35), 446 (44), 315 (28), 84 (100). HREIMS Calcd for $C_{32}H_{44}O_5$: 508.3189. Found: 508.3183. Anal. (Table 1).

Methyl 3β-Hydroxy-11-oxours-12-en-28-oate (47). A solution of methyl 3β-acetoxy-11-oxours-12-en-28-oate (**46**)[10] (150 mg, 0.29 mmol) and KOH (1.0 g) in MeOH (10 mL) was heated under reflux for 30 min. After removal of MeOH in vacuo, the resultant mixture was acidified with 6 M aqueous HCl solution. The aqueous layer was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) (three times). The extract was worked up according to the standard method to give **47** as an amorphous solid (138 mg, quantitative): UV (EtOH) λ_{max} (log ϵ): 250 (4.17) nm. IR (KBr): 3494, 2928, 2869, 1728, 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.59 (1H, s), 3.60 (3H, s), 3.21 (1H, dd, J = 5.9, 10.6 Hz), 2.78 (1H, ddd, J = 3.5, 3.5, 13.6 Hz), 2.41 (1H, d, J = 11.4 Hz), 2.29 (1H, s), 2.07 (1H, m), 1.29, 1.11, 0.99 (each 3H, s), 0.96 (3H, d, J = 6.2 Hz), 0.90 (3H, s), 0.86 (3H, d, J = 6.2 Hz), 0.79 (3H, s). ^{13}C NMR ($CDCl_3$): δ 200.1, 177.4, 163.0, 130.9, 78.9, 61.7, 55.2, 52.9, 52.0, 47.9, 44.8, 43.9, 39.34, 39.28, 38.82, 38.77, 37.3, 36.2, 33.2, 30.5, 28.6, 28.3, 27.5, 24.1, 21.3, 21.2, 19.0, 17.6, 17.3, 16.4, 15.8. EIMS (70 eV) *m/z*: 484 $[M]^+$ (40), 317 (100), 276 (48), 257 (34). HREIMS Calcd for $C_{31}H_{48}O_4$: 484.3553. Found: 484.3552. This material was used for the next reaction without further purification.

Methyl 3,11-Dioxours-12-en-28-oate (48). To a solution of **47** (144 mg, 0.30 mmol) in acetone (14 mL) in an ice bath was added Jones reagent dropwise until the color of the solution changed to pale brown from green. The mixture was stirred at room temperature for 10 min. After removal of acetone, water was added to the resultant mixture. The aqueous mixture was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) (three times). The extract was worked up according to the standard method to give **48** as an amorphous solid (128 mg, 89%): UV (EtOH) λ_{max} (log ϵ): 252 (4.11) nm. IR (KBr): 2949, 2869, 1726, 1709, 1654 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.65 (1H, s), 3.63 (3H, s), 2.96 (1H, ddd, J = 4.2, 7.1, 13.4 Hz), 2.65 (1H, ddd, J = 7.1, 11.2, 15.9 Hz), 2.45 (1H, d, J = 11.5 Hz), 2.40 (1H, s), 2.37 (1H, ddd, J = 4.2, 6.5, 15.9 Hz), 2.10 (1H, ddd, J = 4.6, 14.7, 14.7 Hz), 1.31, 1.26, 1.10, 1.06 (each 3H, s), 0.98 (3H, d, J = 6.3 Hz), 0.95 (3H, s), 0.88 (3H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 217.5, 199.3, 177.4, 163.6, 130.7, 60.9, 55.6, 52.9, 52.1, 47.9, 47.8, 44.7, 44.0, 39.9, 38.8, 36.9, 36.1, 34.4, 32.6, 30.5, 28.6, 26.6, 24.1, 21.6, 21.2, 21.1, 18.9, 17.3, 15.7. EIMS (70 eV) *m/z*: 482 $[M]^+$ (25), 467 (20), 423 (10), 317 (100), 276 (47), 257 (74). HREIMS Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3400. This material was used for the next reaction without further purification.

Methyl 3β-Hydroxy-12-oxooleanan-28-oate (50). 50 was prepared from methyl 3β-acetoxy-12-oxooleanan-28-oate (**49**)[12] according to the same method as for **47** to give a crystalline solid (quantitative): mp 133–135 °C. IR (KBr): 3540, 2945, 2866, 1725, 1698 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.67 (3H, s), 3.18 (1H, dd, J = 5.0, 10.9 Hz), 2.77 (1H, ddd, J = 3.4, 4.2, 13.4 Hz), 2.60 (1H, d, J = 4.2 Hz), 2.14 (2H, m), 1.84 (2H, m), 0.98, 0.96, 0.95, 0.93, 0.89, 0.84, 0.77 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 212.0, 178.6, 78.8, 55.3, 52.0, 49.9, 47.5, 42.1, 41.4, 39.0, 38.7, 38.1, 37.1, 36.4, 34.6, 33.6, 33.1, 32.1, 32.0, 30.8, 28.1, 27.7, 27.2, 23.3, 22.9, 20.7, 18.5, 16.3, 15.5, 15.4. EIMS (70 eV) *m/z*: 486 $[M]^+$ (37), 471 (100), 411 (65), 278 (68), 218 (65). HREIMS Calcd for $C_{31}H_{50}O_4$: 486.3709. Found: 486.3701.

Methyl 3,12-Dioxooleanan-28-oate (51). 51 was prepared from **50** according to the same method as for **48** to give an amorphous solid (98%): IR (KBr): 2948, 2866, 1723, 1702 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.69 (3H, s), 2.80 (1H, ddd, J = 3.7, 4.4, 13.7 Hz), 2.64 (1H, d, J = 4.4 Hz), 2.53 (1H, ddd, J = 7.2, 10.9, 15.9 Hz), 2.40 (1H, ddd, J = 3.8, 7.0, 15.9 Hz), 2.23 (2H, m), 1.09, 1.05, 1.01, 0.983, 0.976, 0.95, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 217.1, 211.4, 178.6, 55.1, 52.0, 49.4, 47.6, 47.5, 42.2, 41.4, 38.8, 36.8, 36.4, 34.6, 34.1, 33.6, 33.1, 32.2, 31.3, 30.8, 27.8, 26.4, 23.3, 22.9, 21.4, 20.7, 19.7, 16.1, 15.0. EIMS (70 eV) *m/z*: 484 $[M]^+$ (4.2), 469 (39), 409 (100), 357 (6.7), 278 (25), 218 (72). HREIMS Calcd for $C_{31}H_{48}O_4$: 484.3553. Found: 484.3544.

Methyl 3β-Hydroxy-12-oxoolean-9(11)-en-28-oate (53). 53 was prepared from methyl 3β-acetoxy-12-oxoolean-9(11)-en-28-oate (**52**)[14] according to the same method as for **47** to give an amorphous solid (97%): UV (EtOH) λ_{max} (log ϵ): 250 (4.03) nm. IR (KBr): 3549, 3382, 2941, 2865, 1717, 1706, 1654, 1644, 1595 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.75 (1H, s), 3.68 (3H, s), 3.21 (1H, dd, J = 4.8, 11.4 Hz), 3.02 (1H, ddd, J = 3.5, 4.6, 13.4 Hz), 2.84 (1H, d, J = 4.6 Hz), 1.23, 1.18, 1.03 (each 3H, s), 0.99 (6H, s), 0.89, 0.83 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.8, 178.6, 178.5, 122.9, 78.2, 52.0, 50.4, 49.6, 47.5, 45.5, 41.9, 40.2, 39.4, 36.6, 36.0, 34.7, 33.5, 33.1, 33.0, 31.7, 30.8, 28.3, 27.7, 24.0, 23.9, 23.3, 22.9, 22.0, 18.2, 15.8. EIMS (70 eV) *m/z*: 484 $[M]^+$ (4.7), 469 (33), 409 (61), 407 (85), 315 (16), 278 (36), 218 (100). HREIMS Calcd for $C_{31}H_{48}O_4$: 484.3553. Found: 484.3553.

Methyl 3,12-Dioxoolean-9(11)-en-28-oate (54). 54 was prepared from **53** according to the same method as for **48** to give an amorphous solid (92%). An analytically pure sample was obtained by flash column chromatography [hexanes–EtOAc (3:1)]: UV (EtOH) λ_{max} (log ϵ): 250 (3.74) nm. IR (KBr): 2944, 2867, 1722, 1708, 1661, 1594 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.80 (1H, s), 3.70 (3H, s), 3.04 (1H, ddd, J = 3.3, 4.9, 13.2 Hz), 2.89 (1H, d, J = 4.9 Hz), 2.66 (1H, ddd, J = 7.2, 10.9, 15.7 Hz), 2.49 (1H, ddd, J = 3.8, 7.1, 15.7 Hz), 2.22 (1H, ddd, J = 3.9, 7.1, 13.4 Hz), 1.31, 1.28, 1.13, 1.09, 1.010, 1.005, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 216.1, 200.3, 178.5, 176.8, 124.2, 52.0, 51.1, 49.7, 47.7, 47.5, 45.6, 42.0, 39.6, 37.2, 36.0, 34.7, 34.3, 33.5, 33.0, 32.2, 31.7, 30.8, 28.3, 26.4, 24.0, 23.8, 23.3, 22.9, 21.8, 21.6, 19.3. EIMS (70 eV) *m/z*: 482 $[M]^+$ (16), 467 (56), 423 (13), 407 (23), 315 (100), 255 (62), 246 (63). HREIMS Calcd for $C_{31}H_{46}O_4$: 482.3396. Found: 482.3392.

3β-Hydroxyolean-9(11)-en-28-oic Acid (57).[35] A mixture of **52** (2.27 g, 4.31 mmol), KOH (22 g), and anhydrous hydrazine (98%) (25 mL) in diethylene glycol (200 mL) was heated under reflux (inside temperature, 165 °C) for 1.5 h. Excess hydrazine was distilled off from the mixture until the inside temperature rose to 215 °C. Then, the mixture was heated under reflux (inside temperature, 215–220 °C) for 6 h. The mixture was poured into water (500 mL). Aqueous HCl solution (6 M) was added to give a precipitate. The precipitate (dry weight, 1.76 g) was filtered and washed with water (several times). The filtrate was extracted with a mixture of CH_2Cl_2 and Et_2O (1:2) (three times). The extract was worked up according to the standard method to give a solid (0.36 g). The combined solids were crystallized from a mixture of CH_2Cl_2 and MeOH (1:1) to afford **57** as colorless crystals (first crop, 670 mg; second crop, 180 mg). The solid obtained from the mother liquid was subjected to flash column chromatography [hexanes–EtOAc (2:1)] to give **57** as crystalline solid (200 mg, total weight: 1050 mg; 53%): mp >275 °C dec. IR (KBr): 3467, 3305, 2947, 2875, 1692 cm^{-1}. ^1H NMR [acetone-d_6, internal standard: δ 2.05 (CD_2HCOCD_3)]: δ 5.35 (1H, t, J = 3.7 Hz), 3.11 (1H, dd, J = 6.8, 9.0 Hz), 1.14, 1.11, 0.98, 0.94, 0.93, 0.89, 0.78 (each 3H, s). EIMS (70 eV) *m/z*: 456 $[M]^+$ (32), 446 (26), 441 (15), 302 (16), 248 (100). HREIMS Calcd for $C_{30}H_{48}O_3$: 456.3603. Found: 456.3603.

3-Oxoolean-9(11)-en-28-oic Acid (58). 58 was prepared from **57** according to the same method as for **48** to give an amorphous solid (95%): IR (KBr): 2947, 2870, 1708, 1694 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.38 (1H, t, J = 3.4 Hz), 2.63 (1H, ddd, J = 7.1, 11.5, 15.9 Hz), 2.42 (1H, ddd, J = 3.7, 6.8, 15.9 Hz), 1.25, 1.13, 1.09, 0.97, 0.94 (each 3H, s), 0.90 (6H, s). ^{13}C NMR ($CDCl_3$): δ 217.9, 185.2, 152.5, 118.7, 52.8, 48.1, 47.7, 43.8, 38.7, 38.6, 38.4, 36.2, 35.7, 34.9, 34.4, 33.7, 33.6, 33.2, 31.8, 30.8, 28.6, 27.3, 26.3, 25.5, 24.9, 23.6, 23.5, 21.5, 19.6, 18.7. EIMS (70 eV) *m/z*: 454 $[M]^+$ (32), 439 (13), 408 (26), 248 (65), 235 (100). HREIMS Calcd for $C_{30}H_{46}O_3$: 454.3447. Found: 454.3439.

Methyl 3-Oxooleana-1,11,13(18)-trien-28-oate (60). 60 was prepared from methyl 3-oxooleana-11,13(18)-dien-28-oate (**59**)[17] according to the same method as for **9**. The crude solid was subjected to flash column chromatography [hexanes–EtOAc (6:1)] to give **60** as a crystalline solid (66%): mp 131–133 °C. UV (EtOH) λ_{max} (log ϵ): 246 (4.54), 252 (4.54) nm. IR (KBr): 3029, 2944, 2859, 1726, 1674 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.27 (1H, d, J = 10.3 Hz), 6.57 (1H, dd, J = 2.9, 10.5 Hz), 5.89 (1H, d, J = 10.3 Hz), 5.79 (1H, dd, J = 1.7, 10.5 Hz), 3.68 (3H, s), 2.54 (1H, d, J = 14.4 Hz), 2.28 (2H, m), 1.91 (1H, m),

1.18, 1.17, 1.10, 0.98, 0.95, 0.86, 0.81 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 205.5, 177.1, 159.1, 136.2, 133.2, 126.7, 125.7, 125.0, 53.4, 52.0, 48.6, 48.4, 45.0, 42.4, 41.7, 40.8, 39.3, 37.0, 35.6, 32.8, 32.4, 32.0, 27.7, 25.2, 24.3, 21.32, 21.27, 20.0, 19.2, 16.7. EIMS (70 eV) *m/z*: 464 $[M]^+$ (84), 449 (13), 405 (100), 327 (14), 267 (19), 239 (29). HREIMS Calcd for $C_{31}H_{44}O_3$: 464.3290. Found: 464.3293.

Methyl 2-Hydroxymethylene-3,12-dioxoolean-9(11)-en-28-oate (62). To a solution of **54** (4.00 g, 8.29 mmol) in dry benzene (90 mL) was added ethyl formate (97%) (3.0 mL) and NaOMe (2.68 g, 50 mmol). The mixture was stirred at room temperature for 2 h. Then the mixture was diluted with a mixture of CH_2Cl_2 and Et_2O (1:2) and washed with 5% aqueous HCl solution (three times). The washings were reextracted with a mixture of CH_2Cl_2 and Et_2O (1:2) and the combined organic layers were worked up according to the standard method to give **62** as an amorphous solid (4.19 g, 99%): UV (EtOH) λ_{max} (log ϵ): 252 (3.66), 294 (3.53) nm. IR (KBr): 3461, 2950, 2867, 1724, 1661, 1596 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 14.86 (1H, d, J = 2.8 Hz), 8.77 (1H, d, J = 2.8 Hz), 5.90 (1H, s), 3.70 (3H, s), 3.05 (1H, ddd, J = 3.1, 4.5, 13.6 Hz), 2.92 (1H, d, J = 4.5 Hz), 2.62 (1H, d, J = 14.4 Hz), 2.30 (1H, d, J = 14.4 Hz), 1.28, 1.24, 1.18, 1.17, 1.02, 1.01, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.3, 190.2, 188.3, 178.5, 175.8, 124.4, 105.1, 52.1, 49.7, 48.4, 47.5, 45.6, 42.0, 40.6, 39.3, 37.2, 36.0, 34.7, 33.5, 33.0, 31.7, 31.5, 30.8, 28.5, 28.4, 23.6, 23.3, 23.2, 22.9, 21.8, 21.0, 19.1. EIMS (70 eV) *m/z*: 510 $[M]^+$ (11), 495 (39), 435 (38), 315 (100), 255 (55). HREIMS Calcd for $C_{32}H_{46}O_5$: 510.3345. Found: 510.3351. This material was used for the next reaction without further purification.

Methyl 12-Oxoisoxazolo[4,5-*b*]olean-9(11)-en-28-oate (63). To a solution of **62** (4.00 g, 7.83 mmol) in EtOH (110 mL) and water (11 mL) was added hydroxylamine hydrochloride (5.44 g, 78 mmol). The mixture was heated under reflux for 1 h. The mixture was concentrated in vacuo and water (50 mL) was added. The mixture was extracted with EtOAc (three times). The combined organic layers were washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a solid. The solid was subjected to flash column chromatography [hexanes−EtOAc (3:1)] to give **63** as an amorphous solid (2.63 g, 66%): UV (EtOH) λ_{max} (log ϵ): 238 (3.63) nm. IR (KBr): 2944, 2867, 1724, 1660, 1596 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.07 (1H, s), 5.89 (1H, s), 3.70 (3H, s), 3.05 (1H, ddd, J = 3.7, 4.6, 13.4 Hz), 2.93 (1H, d, J = 4.6 Hz), 2.79 (1H, d, J = 15.1 Hz), 2.40 (1H, d, J = 15.1 Hz), 1.35, 1.29, 1.27, 1.16, 1.03, 1.01, 0.90 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 200.2, 178.5, 176.3, 172.3, 150.4, 124.7, 108.7, 52.1, 49.9, 49.7, 47.5, 45.8, 42.0, 41.5, 36.1, 35.4, 34.7, 33.8, 33.5, 33.0, 31.7, 31.5, 30.9, 29.0, 28.4, 24.8, 23.29, 23.25, 22.9, 21.8, 21.6, 18.5. EIMS (70 eV) *m/z*: 507 $[M]^+$ (14), 492 (51), 446 (25), 432 (49), 315 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3354.

Methyl 2-Cyano-3,12-dioxoolean-9(11)-en-28-oate (64). To a solution of **63** (2.00 g, 3.94 mmol) in MeOH (60 mL) and Et_2O (125 mL) in an ice bath was added NaOMe (7.25 g, 134 mmol). The mixture was stirred at room temperature for 45 min and then diluted with a mixture of CH_2Cl_2 and Et_2O (1:2). It was washed with 5% aqueous HCl solution (three times) and the acidic washings were reextracted with a mixture of CH_2Cl_2 and Et_2O (1:2). The combined organic layers were worked up according to the standard method to give **64** as an amorphous solid (2.00 g, quantitative): UV (EtOH) λ_{max} (log ϵ): 242 (4.16) nm. IR (KBr): 3411, 2944, 2867, 2206, 1722, 1661, 1636, 1597 cm^{-1}. ^{1}H NMR of major tautomer **64a** ($CDCl_3$): δ 7.08 (1H, brs), 5.75 (1H, s), 3.67 (3H, s), 3.01 (1H, ddd, J = 3.7, 4.6, 13.7 Hz), 2.89 (1H, d, J = 4.6 Hz), 2.40 (1H, d, J = 15.3 Hz), 2.23 (1H, d, J = 15.3 Hz), 1.24, 1.21, 1.19, 1.11 (each 3H, s), 0.98 (6H, s), 0.88 (3H, s). EIMS (70 eV) *m/z*: 507 $[M]^+$ (84), 492 (99), 432 (58), 315 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3340. This material was used for the next reaction without further purification.

Methyl 2-Hydroxymethylene-3,11-dioxoolean-12-en-28-oate (65). **65** was prepared from **45** according to the same method as for **62** to give a crystalline solid (98%): mp 232−234 °C. UV (EtOH) λ_{max} (log ϵ): 254 (4.15), 296 (3.91) nm. IR (KBr): 3456, 2944, 2867, 1728, 1656, 1589 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 14.87 (1H, d, J = 2.7 Hz), 8.62 (1H, d, J = 2.7 Hz), 5.69 (1H, s), 3.64 (3H, s), 3.49 (1H, d, J = 14.8 Hz), 3.03 (1H, dd, J = 3.6, 13.9 Hz), 2.40 (1H, s), 2.05 (1H, ddd, J = 4.1, 13.7, 13.7 Hz), 1.93 (1H, d, J = 14.8 Hz), 1.36, 1.18, 1.12, 1.08, 0.95, 0.94, 0.93 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.9, 189.6, 189.2, 177.6, 169.6, 128.0, 106.0, 59.8, 52.4, 52.1, 46.4, 44.8, 44.5, 43.8, 41.8, 40.2, 39.9, 36.5, 33.8, 33.0, 32.0, 31.7, 30.9, 28.6, 28.0, 23.64, 23.59, 23.1, 21.1, 18.8, 18.7, 14.8. EIMS (70 eV) *m/z*: 510 $[M]^+$ (14), 495 (21), 451 (22), 446 (42), 435 (22), 317 (31), 257 (100). HREIMS Calcd for $C_{32}H_{46}O_5$: 510.3345. Found: 510.3348.

Methyl 11-Oxoisoxazolo[4,5-*b*]olean-12-en-28-oate (66). **66** was prepared from **65** according to the same method as for **63** to give an amorphous solid (74%): UV (EtOH) λ_{max} (log ϵ): 250 (4.10) nm. IR (KBr): 2944, 2867, 1728, 1657, 1624 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.99 (1H, s), 5.71 (1H, s), 3.67 (1H, d, J = 15.5 Hz), 3.64 (3H, s), 3.04 (1H, dd, J = 3.8, 13.6 Hz), 2.51 (1H, s), 2.06 (1H, ddd, J = 4.2, 13.9, 13.9 Hz), 2.03 (1H, d, J = 15.5 Hz), 1.37, 1.31, 1.22, 1.06, 0.96, 0.94, 0.93 (each 3H, s). ^{13}C NMR ($CDCl_3$): δ 199.8, 177.6, 172.4, 169.6, 150.5, 128.1, 109.2, 60.3, 53.5, 52.1, 46.4, 45.1, 44.5, 43.8, 41.8, 38.7, 36.2, 34.9, 33.9, 33.1, 32.1, 31.7, 30.9, 29.1, 28.1, 23.7, 23.6, 23.1, 21.7, 18.7, 18.2, 15.8. EIMS (70 eV) *m/z*: 507 $[M]^+$ (31), 492 (30), 448 (20), 432 (28), 257 (72), 217 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3345.

Methyl 2-Cyano-3,11-dioxoolean-12-en-28-oate (67). **67** was prepared from **66** by the similar method as for **64**. The crude solid was subjected to flash column chromatography [hexanes−EtOAc (2:1)] to give **67** as an amorphous solid (92%): UV (EtOH) λ_{max} (log ϵ): 246 (4.18) nm. IR (KBr): 3411, 2944, 2867, 2200, 1725, 1656 cm^{-1}. ^{1}H NMR of major tautomer **67a** ($CDCl_3$): δ 6.40 (1H, brs), 5.67 (1H, s), 3.62 (3H, s), 3.33 (1H, d, J = 15.9 Hz), 3.02 (1H, dd, J = 3.7, 13.7 Hz), 2.53 (1H, s), 2.36 (1H, d, J = 15.9 Hz), 1.33, 1.15, 1.11, 1.08 (each 3H, s), 0.92 (6H, s), 0.87 (3H, s). EIMS (70 eV) *m/z*: 507 $[M]^+$ (3.7), 492 (5.2), 447 (5.8), 432 (8.4), 276 (7.0), 257 (21), 217 (31), 84 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3349.

Methyl 2-Hydroxymethylene-3,11-dioxours-12-en-28-oate (68). **68** was prepared from **48** according to the same method as for **62** to give an amorphous solid (89%): UV (EtOH) λ_{max} (log ϵ): 254 (4.06), 298 (3.84) nm. IR (KBr): 3454, 2978, 2931, 2866, 1728, 1659, 1619, 1590 cm^{-1}. ^{1}H NMR (500 MHz, by a Varian Unityplus, $CDCl_3$): δ 14.87 (1H, d, J = 3.2 Hz), 8.63 (1H, d, J = 3.2 Hz), 5.67 (1H, s), 3.63 (3H, s), 3.46 (1H, d, J = 14.9 Hz), 2.46 (1H, d, J = 11.2 Hz), 2.40 (1H, s), 2.10 (1H, m), 1.98 (1H, d, J = 14.9 Hz), 1.31, 1.20, 1.13, 1.12 (each 3H, s), 0.98 (1H, d, J = 6.3 Hz), 0.96 (3H, s), 0.88 (3H, d, J = 6.6 Hz). ^{13}C NMR (125.705 MHz, by a Varian Unityplus, $CDCl_3$): δ 199.4, 189.7, 189.2, 177.4, 163.7, 130.9, 106.0, 59.5, 53.0, 52.4, 52.1, 47.9, 44.4, 44.0, 40.2, 40.0, 38.9, 38.8, 36.5, 36.1, 32.2, 30.5, 28.7, 28.6, 24.1, 21.2, 21.1, 18.9, 18.7, 17.3, 14.9. EIMS (70 eV) *m/z*: 510 $[M]^+$ (15), 495 (48), 435 (42), 315 (100), 274 (22), 255 (57). HREIMS Calcd for $C_{32}H_{46}O_5$: 510.3345. Found: 510.3347.

Methyl 11-Oxoisoxazolo[4,5-*b*]urs-12-en-28-oate (69). **69** was prepared from **68** according to the same method as for **63** to give an amorphous solid (81%): UV (EtOH) λ_{max} (log ϵ): 248 (4.09) nm. IR (KBr): 2973, 2937, 2866, 1727, 1658, 1619 cm^{-1}. ^{1}H NMR (500 MHz, by a Varian Unityplus, $CDCl_3$): δ 7.99 (1H, s), 5.68 (1H, s), 3.64 (1H, d, J = 15.6 Hz), 3.63 (3H, s), 2.50 (1H, s), 2.46 (1H, d, J = 11.5 Hz), 2.11 (1H, m), 2.07 (1H, d, J = 15.6 Hz), 1.33, 1.31, 1.23, 1.09 (each 3H, s), 0.98 (3H, d, J = 6.6 Hz), 0.97 (3H, s), 0.89 (3H, d, J = 6.6 Hz). ^{13}C NMR (125.705 MHz, by a Varian Unityplus, $CDCl_3$): δ 199.2, 177.3, 172.4, 163.7, 150.5, 130.8, 109.2, 60.0, 53.5, 52.9, 52.1, 47.8, 44.7, 44.0, 38.9, 38.8, 38.6, 36.2, 36.1, 34.9, 32.3, 30.5, 29.1, 28.7, 24.1, 21.7, 21.2, 21.1, 18.7, 18.2, 17.3, 15.8. EIMS (70 eV) *m/z*: 507 $[M]^+$ (9.3), 492 (13), 317 (13), 257 (24), 217 (12), 84 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3351.

Methyl 2-Cyano-3,11-dioxours-12-en-28-oate (70). 70 was prepared from **69** by the similar method as for **64**. The crude solid was subjected to flash column chromatography [hexanes–EtOAc (2:1)] to give **70** as a crystalline solid (94%): mp 169–171 °C. UV (EtOH) λ_{max} (log ϵ): 246 (4.17) nm. IR (KBr): 3401, 2978, 2937, 2866, 2202, 1725, 1668 cm^{-1}. ^1H NMR of major tautomer **70a** (500 MHz, by a Varian Unityplus, $CDCl_3$): δ 5.86 (1H, brs), 5.66 (1H, s), 3.62 (3H, s), 3.33 (1H, d, J = 15.7 Hz), 2.45 (1H, d, J = 10.3 Hz), 2.33 (1H, s), 2.10 (1H, m), 1.92 (1H, d, J = 15.7 Hz), 1.29, 1.17, 1.15, 1.09 (each 3H, s), 0.97 (3H, d, J = 6.4 Hz), 0.93 (3H, s), 0.87 (3H, d, J = 6.6 Hz). EIMS (70 eV) *m/z*: 507 $[M]^+$ (25), 492 (31), 467 (45), 446 (54), 317 (34), 276 (26), 257 (85), 217 (100). HREIMS Calcd for $C_{32}H_{45}O_4N$: 507.3349. Found: 507.3351.

Methyl 3-Hydroxy-2-methoxycarbonyl-12-oxooleana-2,9(11)-dien-28-oate (71). A mixture of **54** (258 mg, 0.53 mmol) and 1.8 M DMF solution of methoxymagnesium methyl carbonate (Stiles' reagent) (2.5 mL, 4.5 mmol) was heated at 110 °C for 1 h while a slow stream of N_2 was bubbled through the mixture with a pipet. To the mixture were added 5% aqueous HCl solution and EtOAc. The aqueous layer was extracted with EtOAc (three times). The combined organic layers were washed with water (three times) and saturated aqueous NaCl solution (three times), dried over $MgSO_4$, and filtered. The filtrate was evaporated in vacuo to give a solid (305 mg). To a solution of the solid in THF (6 mL) was added excessive amount of ethereal diazomethane. The mixture was kept at room temperature for 10 min. The mixture was evaporated in vacuo to give a solid (310 mg). The solid was subjected to flash column chromatography [hexanes–EtOAc (4:1)] to give **71** as crystals (225 mg, 78%): mp 210–211 °C. UV (EtOH) λ_{max} (log ϵ): 252 (4.20) nm. IR (KBr): 2944, 2867, 1725, 1661, 1618 cm^{-1}. ^1H NMR ($CDCl_3$): δ 12.49 (1H, s), 5.94 (1H, s), 3.76, 3.69 (each 3H, s), 3.04 (1H, ddd, J = 3.1, 4.9, 13.2 Hz), 2.90 (1H, d, J = 4.9 Hz), 2.70 (1H, d, J = 15.3 Hz), 2.06 (1H, d, J = 15.3 Hz), 1.26, 1.20, 1.17, 1.14 (each 3H, s), 1.00 (6H, s), 0.89 (3H, s). ^{13}C NMR ($CDCl_3$): δ 200.5, 178.5, 176.9, 176.7, 173.9, 124.5, 94.1, 52.0, 51.8, 49.7, 48.6, 47.5, 45.6, 42.0, 39.3, 38.6, 36.3, 36.1, 34.7, 33.5, 33.1, 31.7, 31.5, 30.8, 28.6, 28.4, 24.3, 23.3, 23.2, 22.9, 21.8, 20.4, 19.1. EIMS (70 eV) *m/z*: 540 $[M]^+$ (3.9), 525 (5.7), 508 (23), 493 (54), 433 (35), 315 (100). HREIMS Calcd for $C_{33}H_{48}O_6$: 540.3451. Found: 540.3454.

Evaluation Methods. 1. Reagents. Recombinant mouse IFN-γ (LPS content, <10 pg/mL) was purchased from Genzyme (Cambridge, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Inhibitory test compounds were dissolved in DMSO before addition to cell cultures; final concentrations of DMSO were 0.1% or less. Controls with DMSO alone were run in all cases.

2. Cell Culture. To obtain primary macrophages, female CD-1 mice, 5–10 weeks of age (Charles River Breeding Laboratories, Wilmington, MA), were injected intraperitoneally with 2 mL of 4% thioglycollate broth (Difco Laboratories, Detroit, MI). Four days after injection, peritoneal macrophages were harvested and processed according to Nathan's procedure.[4b] Cells were seeded in 96-well plates at 2 × 10^5 cells/well and incubated for 48 h with 20 ng/mL IFN-γ in the presence or absence of inhibitory test compounds

3. Measurement of NO Production in Mouse Macrophages. Nitrite accumulation was used as an indicator of NO production in the medium and was assayed by the Griess reaction.[4a] Griess reagent (100 μL) was added to 100 μL of each supernatant from IFN-γ or inhibitory test compound-treated cells in triplicate. The protein determination was performed by Bradford protein assay. The plates were read at 550 nm against a standard curve of sodium nitrite.

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A Synthetic Triterpenoid, 2-Cyano-3,12-dioxooleana-1,9-dien-28-oic Acid (CDDO), Is a Ligand for the Peroxisome Proliferator-Activated Receptor γ

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A novel synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), previously reported to have potent differentiating, antiproliferative, and antiinflammatory activities, has been identified as a ligand for the peroxisome proliferator-activated receptor γ (PPARγ). CDDO induces adipocytic differentiation in 3T3-L1 cells, although it is not as potent as the full agonist of PPARγ, rosiglitazone. Binding studies of CDDO to PPARγ using a scintillation proximity assay give a K_i between 10^{-8} to 10^{-7} M. In transactivation assays, CDDO is a partial agonist for PPARγ. The methyl ester of CDDO, CDDO-Me, binds to PPARγ with similar affinity, but is an antagonist. Like other PPARγ ligands, CDDO synergizes with a retinoid X receptor (RXR)-specific ligand to induce 3T3-L1 differentiation, while CDDO-Me is an antagonist in this assay. The partial agonism of CDDO and the antagonism of CDDO-Me reflect the differences in their capacity to recruit or displace cofactors of transcriptional regulation; CDDO and rosiglitazone both release the nuclear receptor corepressor, NCoR, from PPARγ, while CDDO-Me does not. The differences between CDDO and rosiglitazone as either partial or full agonists, respectively, are seen in the weaker ability of CDDO to recruit the coactivator CREB-binding protein, CBP, to PPARγ. Our results establish the triterpenoid CDDO as a member of a new class of PPARγ ligands. (Molecular Endocrinology 14: 1550–1556, 2000)

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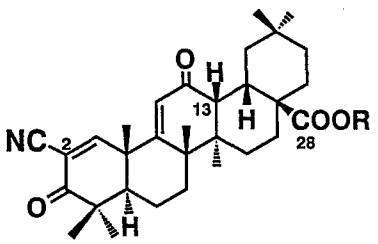
INTRODUCTION

Triterpenoids are a large family of structures synthesized in plants through the cyclization of squalene and have been used in traditional Asian medicine for centuries (1). Naturally occurring triterpenoids like oleanolic acid (OA) and ursolic acid (UA) are known to have relatively weak antiinflammatory and anticarcinogenic activities (2, 3). To increase their usefulness, we have synthesized a series of novel derivatives of OA and UA and have shown that some derivatives of OA are much more potent than the parent compound in suppressing the induction of the enzymes, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (4–6). The most active of these synthetic derivatives, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) (Fig. 1), is not only antiinflammatory, but also has potent antiproliferative and differentiating activities (7, 8).

One of the effects of CDDO on differentiation can be easily measured by its ability to convert 3T3-L1 fibroblasts into mature adipocytes (8). These fibroblasts un-

dergo dramatic morphological and biochemical changes upon induction of differentiation and accumulate triglyceride (9). The classic inducers for this process have been a combination of 1-methyl-3-isobutyl xanthine, dexamethasone, and insulin (MDI), although more recently, ligands for the peroxisome proliferator-activated receptor γ (PPARγ) such as the thiazolidinedione, rosiglitazone, have also been identified as potent inducers of adipogenic differentiation (10–12).

PPARγ is a member of the nuclear receptor superfamily of transcription factors. It forms heterodimers with the retinoid X receptor (RXR) to activate gene transcription (13–15). This cooperation is reflected in the ability of PPARγ and RXR ligands to synergize in the induction of adipocyte differentiation (16). Furthermore, binding of ligands to nuclear receptors such as PPARγ results in the recruitment or displacement of different cofactors that either enhance or suppress transcription (17). In particular, the binding of an agonist to nuclear receptors results in the recruitment of coactivators such as NCoA/SRC-1 (nuclear receptor coactivator/steroid receptor coactivator-1) and p300/CBP (CREB binding protein) and leads to activation of transcription (18, 19). In contrast, corepressors such as NCoR (nuclear receptor corepressor) or SMRT (silencing mediator for retinoid and thyroid hormone receptors) can suppress transcription by binding to receptors either in the absence of their ligands or when an antagonist is bound (20, 21).



R = H (CDDO)
R = CH_3 (CDDO-Me)

Fig. 1. Chemical Structures of CDDO and CDDO-Me

Here we demonstrate that the adipogenic effect of CDDO is due to its binding to PPARγ. It not only induces differentiation as a single agent, but also acts synergistically with an RXR-specific ligand. Binding and transactivation studies indicate that CDDO is a partial agonist for PPARγ. We also report that the C-28 methyl ester of CDDO, CDDO-Me (6, 7), is a PPARγ antagonist, and that these opposite activities of CDDO and CDDO-Me can be explained by their differential effects on the interactions of cofactors with PPARγ.

RESULTS

CDDO Induces Differentiation in 3T3-L1 Cells

To induce adipocytic differentiation, 3T3-L1 fibroblasts were treated with MDI mix (Fig. 2B), or rosigli-

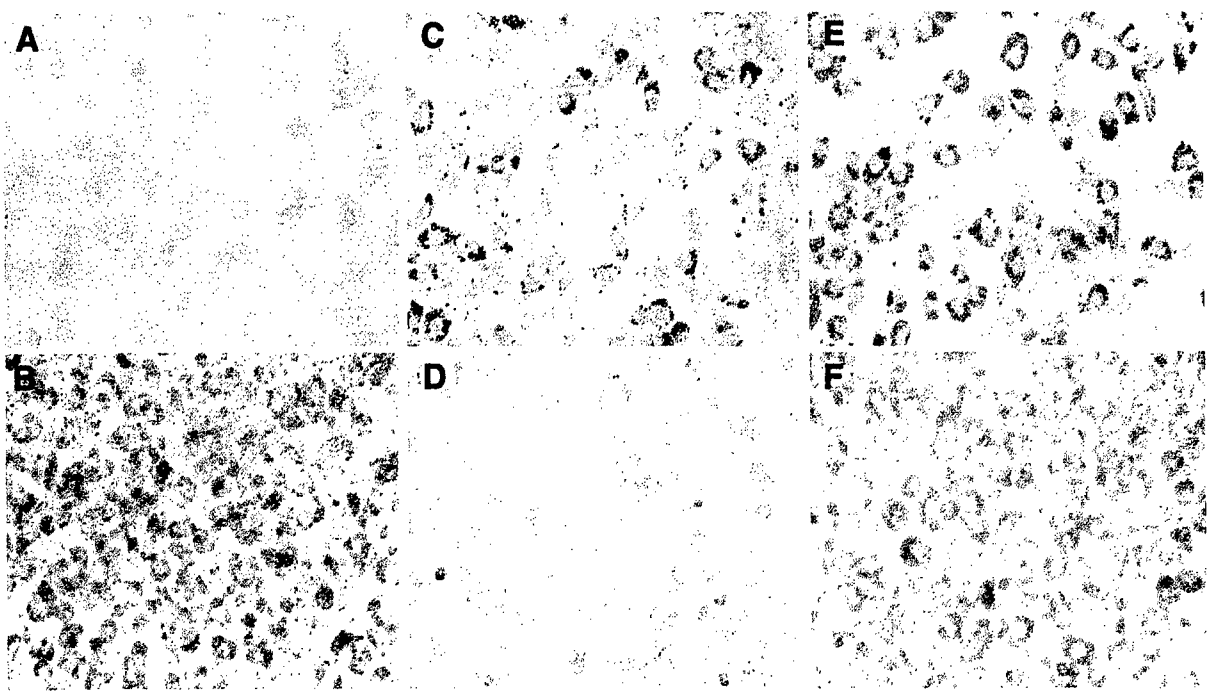


Fig. 2. CDDO Induces Accumulation of Triglyceride in 3T3-L1 Cells

3T3-L1 cells were differentiated as described in *Materials and Methods* and stained on day 6. Triglyceride was stained with Oil Red O, and nuclei were counterstained with hematoxylin. A, Cells maintained in DMEM/10% FBS for 6 days. B–F, Cells differentiated with MDI (B), 0.1 μM (C) or 1 μM (D) CDDO, 0.1 μM (E) or 1 μM (F) rosiglitazone.

tazone at 100 nM (Fig. 2E) or 1 μM (Fig. 2F) for 2 days. Accumulation of triglyceride droplets was evident on the sixth day, as shown by positive staining with Oil Red O. Treatment with CDDO (100 nM), however, induced differentiation more slowly and less effectively (Fig. 2C); the percentage of differentiated cells was approximately 30% by day 6 (Fig. 2C) and peaked at 50% by day 8 (not shown). Interestingly, unlike rosiglitazone at 1 μM (Fig. 2F), a higher dose of CDDO (1 μM) was not effective (Fig. 2D), even when evaluated at day 10. In fact, this higher dose was inhibitory to differentiation induced by MDI or rosiglitazone (data not shown).

To quantify the degree of differentiation, the enzyme glycerol-3-phosphate dehydrogenase (GPDH), a key enzyme in triglyceride synthesis, was used as a marker (22). GPDH activity correlated well with visual detection of triglyceride droplets under the light microscope. Cells treated with CDDO were assayed for GPDH activity on day 8 while those treated by rosiglitazone or MDI were assayed on day 6, as shown in Fig. 3A, which confirms that CDDO is a weaker inducer than MDI or rosiglitazone, and that it has no adipogenic activity at 1 μM. The C-28 methyl ester of CDDO, CDDO-Me, did not induce differentiation in 3T3-L1 cells at all concentrations tested on day 8 (Fig. 3B). Furthermore, it acted in a dose-dependent manner as an antagonist and inhibited differentiation induced by 100 nM rosiglitazone (Fig. 3B). Even though CDDO also inhibits differentiation at 1 μM, at concentrations where it acted as a differentiating agent (100 nM or lower), it did not inhibit differentiation induced by rosiglitazone and its activity was additive to that of the full agonist (data not shown).

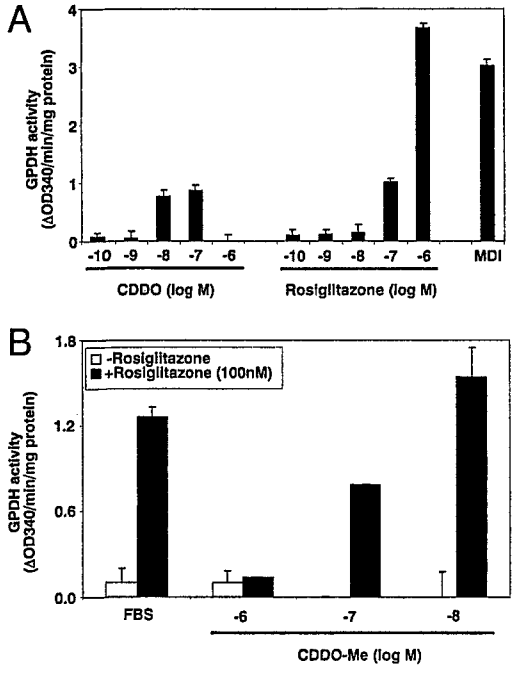


Fig. 3. CDDO Induces GPDH Activity in 3T3-L1 Cells

A, GPDH activities for cells differentiated with CDDO (0.1 nM to 1 μM), rosiglitazone (0.1 nM to 1 μM), or MDI. Cells treated with CDDO were harvested on day 8, while those treated by rosiglitazone and MDI were harvested on day 6. B, GPDH activities for cells differentiated with CDDO-Me (10 nM to 1 μM), in the absence or presence of 100 nM rosiglitazone, assayed on day 6.

CDDO Binds to and Transactivates PPARγ

The adipogenic effect of CDDO suggested that it might be a ligand for PPARγ. Therefore, binding studies were performed using a scintillation proximity assay (SPA), which has been successfully used in the study of PPARs and their ligands (23). Using this assay, CDDO and rosiglitazone were shown to compete for bound ^{3}H-CDDO, with K_i values of 310 nM and 50 nM, respectively (Fig. 4). Importantly, the presence of dithiothreitol (DTT) in the binding buffer interfered with CDDO binding to PPARγ. We repeated these experiments using ^{3}H-rosiglitazone as the ligand and nonradioactive CDDO or CDDO-Me as competitors. Again, the presence of DTT blocked the ability of either CDDO or CDDO-Me to compete for binding to PPARγ; the K_i values in this assay were determined to be 12 nM for CDDO and 130 nM for CDDO-Me (Fig. 5 and Table 1). Both triterpenoids were also tested for binding to PPARα, either in the presence or absence of DTT, and neither binds to PPARα (Table 1).

To determine whether bound CDDO can transactivate PPARγ, a Gal4-PPARγ chimeric protein was used

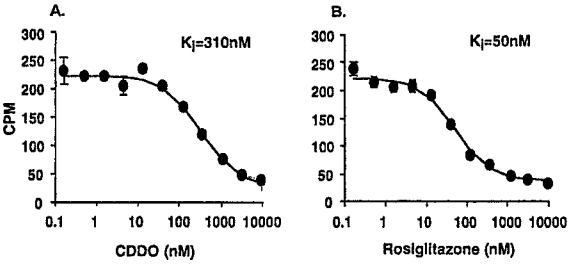


Fig. 4. CDDO Binds to PPARγ

Nonradioactive CDDO (A) or rosiglitazone (B) was used to compete for binding to PPARγ using 50 nM ^{3}H-CDDO as the ligand. The assays were performed in the absence of 10 mM DTT.

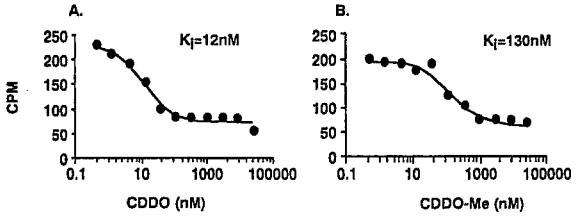


Fig. 5. CDDO and CDDO-Me Compete with Rosiglitazone for Binding to PPARγ

Nonradioactive CDDO (A) or CDDO-Me (B) was used to compete for binding to PPARγ using ^{3}H-rosiglitazone as the ligand. The assays were performed in the absence of 10 mM DTT.

Table 1. K_i Values for CDDO and CDDO-Me Competing for Binding to PPARα or PPARγ, Using ^{3}H-GW2331 (35) and ^{3}H-Rosiglitazone as Ligands, Respectively

Compounds	hPPARα (K_i, nM)		hPPARγ (K_i, nM)	
	+DTT	−DTT	+DTT	−DTT
CDDO	3,000–100,000	7,600	1,000–25,000	12
CDDO-Me	1,500–21,000	12,000	1,000–13,000	130

The assays were performed in the absence or presence of 10 mM DTT.

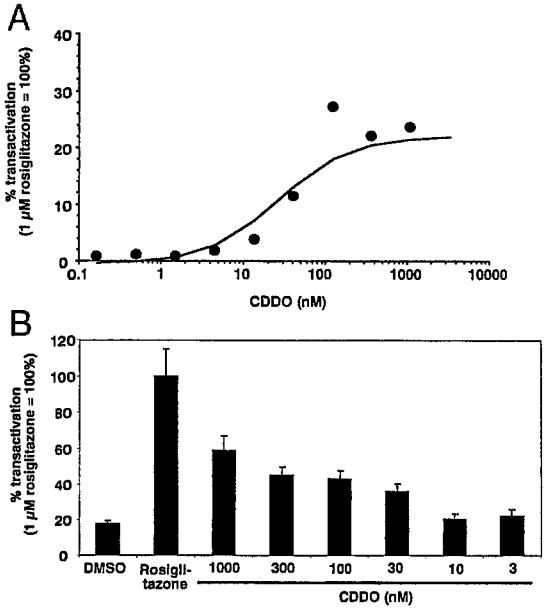


Fig. 6. CDDO Activates PPARγ-Driven Transcription

A, CV-1 cells were transfected with the Gal4-PPARγ chimeric protein as described previously (28). Activity of the reporter, SPAP, was measured by absorbance at 405 nM. B, HeLa cells were transfected with wild-type PPARγ and a luciferase construct driven by a PPRE derived from the acyl-CoA oxidase gene promoter. In both panels A and B, reporter activities, normalized against β-gal, are expressed in reference to that found for 1 μM rosiglitazone (100%).

to drive the expression of secreted placental alkaline phosphatase (SPAP) linked to the DNA binding sequence of Gal4. Figure 6A shows that CDDO transactivates Gal4-PPARγ in a dose-dependent manner, although the maximal level of transactivation achieved by CDDO was only 26% of that obtained with rosiglitazone (1 μM). We also tested the ability of CDDO to transactivate the wild-type PPARγ receptor in the context of a natural PPARγ response element (PPRE) derived from the acyl-CoA oxidase gene promoter (13). CDDO had 57% of the maximal activity obtained with 1 μM rosiglitazone in this system (Fig. 6B). CDDO-Me, which also bound to PPARγ with high affinity, did not transactivate PPARγ in either system (data not shown). To ensure the specificity of this transactivation, CDDO, rosiglitazone, and another PPARγ ligand, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) (24, 25), were tested in a transactivation assay for PPARα. While the PPARα ligand Wy14,643 transactivated this receptor, none of the PPARγ ligands did (data not shown). This result is consistent with the fact that CDDO does not bind to PPARα (Table 1).

CDDO Synergizes with an RXR-Specific Ligand to Induce 3T3-L1 Differentiation

The above data demonstrate that CDDO is a partial agonist for PPARγ. Since PPARγ is known to heterodimerize with RXR and activate transcription (13, 26), we determined if CDDO would synergize with the RXR-specific ligand LG100268 (27). Figure 7 shows that although LG100268 alone at 1 μM induced only slight differentiation in 3T3-L1 cells, it greatly potentiates the activity of CDDO. In contrast, not only did CDDO-Me fail to synergize with LG100268 to induce differentiation, it inhibited the differentiation induced by the RXR ligand (Fig. 7). Unlike the GPDH assays for CDDO in Fig. 3A, this experiment was performed on day 6 to minimize the differentiating effect of CDDO and maximize the level of synergism between CDDO and LG100268.

CDDO and CDDO-Me Differentially Recruit Cofactors to PPARγ

To further explore the mechanisms of action of CDDO and CDDO-Me, a mammalian two-hybrid system was used to examine the ability of CDDO or CDDO-Me to recruit the coactivator, CBP, to PPARγ or to release the corepressor, NCoR, from it; rosiglitazone is known to have both of these activities (28). Figure 8A shows that rosiglitazone recruits CBP to PPARγ in a dose-dependent manner, as expressed by the level of expression of the reporter gene chloramphenicol acetyltransferase (CAT) normalized against β-gal activity.

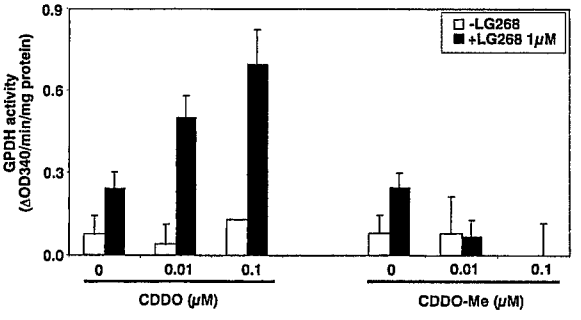


Fig. 7. CDDO Synergizes with LG100268 to Induce 3T3-L1 Differentiation

3T3-L1 cells were differentiated and GPDH activity was assayed as described in *Materials and Methods*. Shown are GPDH activities of cells differentiated with CDDO or CDDO-Me (0.01 μM or 0.1 μM), in the absence or presence of 1 μM LG100268, assayed on day 6 (different from those obtained for CDDO in Fig. 3A, which was done on day 8).

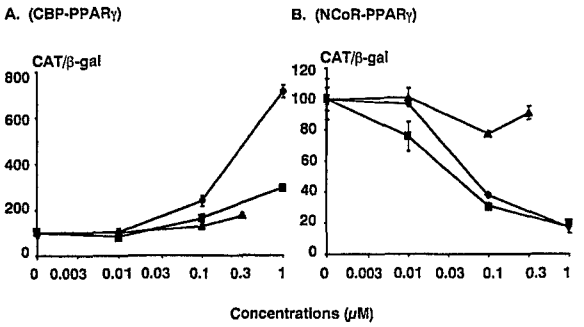


Fig. 8. CDDO and CDDO-Me Differentially Recruit Cofactors

COS-1 cells were transfected as described in *Materials and Methods*. Without ligands, there is little interaction between CBP with PPARγ, but the association between NCoR and PPARγ is strong (28). For comparison purposes, these figures are expressed using the normalized CAT activities of those without ligands as 100. A, Dose-dependent recruitment of CBP to PPARγ by rosiglitazone (♦), CDDO (■), or CDDO-Me (▲). CDDO-Me was toxic to COS-1 cells at 1 μM, so the highest dose used was 0.3 μM. B, Effects of different doses of the same ligands on NCoR/PPARγ interaction.

CDDO also recruits CBP to PPARγ in a dose-dependent manner, but much less so than rosiglitazone. CDDO-Me is also a weaker recruiter of CBP in the concentrations tested. A maximum of 0.3 μM CDDO-Me was used since 1 μM CDDO-Me was toxic to the COS-1 cells used in the transfection assay. We then tested the ability of PPARγ, when bound with CDDO and CDDO-Me, to interact with the corepressor NCoR. Unlike coactivators, the two-hybrid system indicates that NCoR interacts with PPARγ in the absence of ligands (28). When rosiglitazone was added, however, NCoR was released from PPARγ in a dose-dependent manner (Fig. 8B), leading to a decrease in CAT reporter expression. Interestingly, CDDO, although only a partial agonist, was equally capable of releasing NCoR from PPARγ (Fig. 8B). CDDO-Me, which does not transactivate PPARγ, did not lead to a dissociation of the corepressor (Fig. 8B).

DISCUSSION

Previous studies have shown that CDDO is a multifunctional agent, with marked antiinflammatory, antiproliferative, and differentiating activities, as shown by studies in a wide variety of cells (8). It is therefore important to understand the mechanisms of action of this molecule. Although the present studies do little to elucidate the antiinflammatory and antiproliferative activities of CDDO, they do provide the first data that explain some of its ability to control cell differentiation, at least in the context of the conversion of 3T3-L1 fibroblasts to adipocytes. We have shown that CDDO is an effective agent for adipogenic conversion of 3T3-L1 fibroblasts, although it is less active than a prototypical PPARγ ligand such as rosiglitazone. Binding competition assays, using labeled CDDO or rosiglitazone, indicate that CDDO is a ligand for PPARγ, and that this binding could transactivate both the Gal4-PPARγ chimeric and wild-type receptor. The functional interaction of CDDO with PPARγ has been further confirmed by the ability of CDDO to synergize with a ligand specific for RXR; RXR and PPARγ are known to form functional heterodimers (13). Further studies on cofactor interactions are consistent with the observation that CDDO is a partial agonist for PPARγ and that its methyl ester is an antagonist.

Two interesting observations in this study warrant further discussion. One is the biphasic dose response of CDDO in the induction of 3T3-L1 differentiation. At 1 μM, CDDO not only failed to induce differentiation (Fig. 3A), but it could also inhibit those induced by all other known inducers tested, including MDI, rosiglitazone, or RXR-specific ligands (data not shown); the mechanism of this inhibition is unknown. However, based on our studies of CDDO in different biological systems (8), CDDO was shown to be a multifunctional molecule and could be interacting with cellular targets other than PPARγ to inhibit the differentiation process. This characteristic is not unique to CDDO. Recent studies of another well known PPARγ ligand, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), indicate the presence of other cellular targets, namely components of the nuclear factor-κB (NF-κB pathway), for this prostaglandin (29, 30). The antiinflammatory activities of 15d-PGJ_2, in terms of its ability to suppress reporter expression driven by NF-κB or AP-1 elements, have been shown to be dependent on PPARγ (30).

The second observation is the different binding conditions CDDO and rosiglitazone require in the *in vitro* binding studies. Unlike the results obtained with rosiglitazone, the presence of DTT interfered with the binding of CDDO to PPARγ. Due to the presence of an α,β-unsaturated carbonyl function in the A-ring of CDDO, we searched for direct adduct formation between CDDO and DTT but found none. Although we could demonstrate no covalent bond formation between CDDO and DTT, it is still possible that a reversible noncovalent interaction exists. Again, this sensitivity to DTT is not unique to CDDO. 15d-PGJ_2 has also been shown to be sensitive to thiol groups found in DTT or cysteine (29, 30), although there is no convincing chemical evidence to support the notion that a covalent adduct is found between 15d-PGJ_2 and these agents.

The molecular coordinates of the interaction of CDDO with PPARγ remain to be determined. It would appear that a free –COOH group at C-28 is important for agonistic activity in the 3T3-L1 cells, since the methyl ester of CDDO acts as an antagonist in this system. Thus, in 3T3-L1 cells, we have shown that CDDO-Me can block the differentiating effects of rosiglitazone and the RXR-specific ligand, LG100268, as well as those of CDDO itself (data not shown). Although CDDO-Me binds to PPARγ, it does not transactivate the receptor, which may be the result of its

failure to cause release of a corepressor such as NCoR. Given the fact that CDDO is also an inhibitor of differentiation at 1 μM, the mechanisms of the inhibitory actions of CDDO-Me at the same concentration could be attributed to either a direct antagonism of PPARγ, other mechanisms independent of this receptor, or both. It is also important to note that at concentrations higher than 1 μM, CDDO-Me becomes toxic to many cells and thus should not be used at those doses to attribute the activities to the antagonism of PPARγ.

Although the results we described here provide a reasonable explanation for the differentiating effects of CDDO on 3T3-L1 cells, they do not account for other notable activities of CDDO, particularly its ability to suppress the expression of the enzyme iNOS in macrophages. Neither do they explain the ability of CDDO to act as a potent antiproliferative agent on a wide variety of tumor cells or to induce differentiation in leukemia cells. Thus, we have found that while CDDO can suppress iNOS expression in macrophages at doses below 1 nM, a number of PPARγ ligands, including rosiglitazone and 15d-PGJ_2, are inactive in this assay at concentrations below 1 μM (our unpublished data and Refs. 31 and 32). Furthermore, unlike CDDO, thiazolidinediones such as rosiglitazone do not induce differentiation in leukemia cells (our unpublished data). Given the diverse biological activities of CDDO in these systems, we are therefore left with the conclusion that it is likely that another functional receptor system (or systems) beyond PPARγ remain to be identified for CDDO, if we wish to understand the mechanism of action of this agent in cells other than 3T3-L1. The identification of PPARγ as a receptor for CDDO represents the first important step in our understanding of the actions of CDDO, but it is only a beginning in this intriguing problem.

MATERIALS AND METHODS

Reagents and Plasmids

The synthesis of CDDO and its methyl ester have been described previously (7). ^{3}H-CDDO (6 Ci/mmol) was prepared by tritium exchange at the C-13 position with tritium oxide in the presence of triethylamine in chloroform, and the synthesis of ^{3}H-rosiglitazone (26 Ci/mmol) has been described previously (33). pCMX-mPPARα, pCMX-mPPARγ1, PPREx3-tk-Luc (13); pSG5-Gal4-mPPARα-LBD, pCMX-Gal4-mPPARγ1-LBD, MLH100x4-tk-Luc (25); and Gal4-CBP, Gal4-NCoR, VP16-PPARγ2 (28) have been previously described. 1-Methyl-3-isobutyl xanthine, dexamethasone, β-nicotinamide adenine dinucleotide (NADH), and dihydroxyacetone phosphate (DHAP) were obtained from Sigma (St. Louis, MO). Insulin was purchased from Biofluids (Rockville, MD). LG100268 was obtained from Dr. Richard Heyman (Ligand Pharmaceuticals, Inc., San Diego, CA). Reagents for SPA assays have been described (34).

3T3-L1 Differentiation and Analysis

3T3-L1 cells were obtained from Dr. Gustav Lienhard (Dartmouth Medical School, Hanover, NH). Cells were propagated in DMEM/5% calf serum (CS) and differentiated in DMEM/10% FBS. Cells grown to confluency (day −2) were kept for two more days before agents were added (day 0). For MDI treatment, 0.5 mM 1-methyl-3-isobutyl xanthine, 0.25 μM dexamethasone, and 0.35 μM insulin were used for 2 days. Cells were then cultured in DMEM/10% FBS/insulin for the rest of the differentiation process. All other treatments are for day 0 to day 2 only, and medium was changed every 2 days. For Oil Red O staining, cells were fixed in 10% formaldehyde for 1 h and stained with Oil Red O for 2 h. The nuclei were counterstained with hematoxylin and photographed. Lysis buffer for GPDH analysis includes 50 mM Tris, pH 8, 100 mM NaCl, 0.5% NP-40, 1 mM DTT and was supplemented with 1 mM phenylmethylsulfonylfluoride, 10 μg/ml each of leupeptin and aprotinin. GPDH enzyme activity was measured as the consumption of 0.2 mM NADH at 340 nM using 0.2 mM DHAP as the substrate (22).

Transfection Assays

For Gal4-PPARγ transactivation studies, CV-1 cells were transfected as described previously (28). Wild-type PPARγ transfections were performed in HeLa cells using Lipofectamine Plus (Life Technologies, Inc., Gaithersburg, MD) according to manufacturer's instructions. Percentage of transactivation was normalized against 1 μM rosiglitazone. For mammalian two-hybrid assays, COS-1 cells in 24-well plates were transfected using Lipofectamine Plus. Twenty nanograms of CMX-β-gal, 60 ng pG5-CAT, 60 ng VP16-PPARγ2, and 60 ng Gal4-cofactors were used for each well. Ligands were added 4 h after transfection; CAT and β-gal activities were measured 40 h later.

SPA Binding Assays

The details of SPA assays have been published elsewhere (34). In brief, human PPARγ ligand-binding domain was expressed in *Escherichia coli* as a polyhistidine-tagged fusion protein. The protein was purified, biotinylated, and immobilized on streptavidin-modified SPA beads. DTT was washed away and binding assays were performed in 50 mM HEPES, pH 7, 50 mM KCl, 5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS), and 0.1 mg/ml BSA. When DTT was used, its concentration was 10 mM.

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A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production

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Abstract

New oleanane triterpenoids with various substituents at the C-17 position of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) and methyl 2-carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate were synthesized. Among them, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile shows extremely high inhibitory activity (IC_{50} = 1 pM level) against production of nitric oxide induced by interferon-γ in mouse macrophages. This potency is about 100 times and 30 times more potent than CDDO and dexamethasone, respectively.

Introduction

In previous papers, we reported that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**1**), its methyl ester **2** and methyl 2-carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate (**3**) show high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50} = 0.1 nM level).[1-4] We also reported that CDDO is a potent, multifunctional agent in various in vitro assays.[5] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. CDDO also inhibits proliferation of many human tumor cell lines, and blocks *de novo* synthesis of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. The above potencies have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture. Mechanism studies revealed that CDDO is a ligand for peroxisome proliferator–activated receptor γ (PPARγ)[6] and induces apoptosis in human myeloid leukemia cells.[7]

Modifications of rings A and C of oleanolic acid (**30**), a commercially available naturally occurring triterpene, led to the synthesis of CDDO. However, we had not modified the carboxyl group at C-17 of CDDO, which is very important from the perspective of structure–activity relationships (SAR). Because the synthesis of CDDO involves 11 steps from oleanolic acid, this has limited the preparation of sufficient quantities of CDDO to allow such modifications. However, we have recently produced a sufficient amount to be able to synthesize various CDDO derivatives with modified carboxyl groups (i.e., nitrile, esters, glycosides, and amides) at C-17 (see Table 1). As a result, we found that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile (**4**) shows extremely high inhibitory activity (IC_{50} = 1 pM level) against production of NO in mouse macrophages. This potency is about 100 times and 30 times more potent than that of CDDO and dexamethasone, respectively. In this communication, we report the synthesis, inhibitory activity and SAR of these new analogues.

Chemistry

Dinitrile **4** was synthesized from CDDO by the method as shown in Scheme 1. Oxalyl chloride gave acyl chloride **31** in quantitative yield. Amide **15** was prepared in 91% yield from **31** with ammonia gas in benzene. Dehydration of **15** with thionyl chloride gave **4** in 89% yield.[8] Because the C-17 carboxyl group of CDDO is hindered, esterifications of CDDO with alcohols under acidic conditions were not successful. We found that a nucleophilic substitution method using an alkyl halide and DBU in toluene (reflux)[9] gives esters **6** and **9–12** from CDDO in good yield (see Table 1). Allyl ester **8** was successfully prepared in 83% yield from allyl bromide and CDDO using a phase-transfer catalyst.[10] Amides **16–29** were synthesized in good yield by condensation reactions (Methods C–D, see Scheme 1) between acyl chloride **31** and the corresponding amines. Tetra-*O*-acetyl-β-D-glucopyranoside **13** was prepared in 75% yield from tetra-*O*-acetyl-α-D-glucopyranosyl bromide[11] and CDDO using a phase-transfer catalyst.[12] Because in the ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of **13** the anomeric proton was observed at δ 5.70 ppm (1H, d, J = 7.8 Hz), the proton was assigned the β-configuration. Acetyl groups of **13** were removed with saturated ammonia methanol solution to afford β-D-glucopyranoside **14** in 83% yield (Scheme 2). In addition to these CDDO derivatives, we have synthesized derivatives of compound **3**, nitrile **5** and ethyl ester **7** (Scheme 3). Their syntheses require many more steps than the syntheses of CDDO derivatives because the carboxyl group at C-2 must be introduced after the carboxyl group at C-17 is modified. Acid **33** was prepared in 83% yield by cleavage of the known methyl ester **32**[1,4] with LiI in DMF.[13] The same sequence as for **4** gave nitrile **34** in 25% yield (chlorination, 100%; amidation, 100%; and dehydration, 25%). The desired nitrile **5** was synthesized in 4 steps from **34** (yield, 24%) according to the known synthetic sequence for **3**[2,4] (insertion of carboxyl group at C-2 of **34** with Stiles' reagent[14], followed by methylation with diazomethane, 48%; insertion of double bond at C-1 with phenylselenenyl chloride–pyridine and subsequent H_2O_2 oxidation,[15] followed by selective hydrolysis of the C-2 methyl ester with KOH in aqueous methanol, 51%). Ethyl ester **35** was prepared in 99% yield by ethyl iodide and DBU in

toluene. The desired ethyl ester **7** was synthesized in 57% yield from **35** by the same sequence as for **5**.

Biological Results and Discussion

The inhibitory activities [IC_{50} (nM) value] of new synthetic triterpenoids **4–29**,[16] oleanolic acid, and dexamethasone on NO production induced by IFN-γ in mouse macrophages[17] are shown in Table 1. Dinitrile **4** shows extremely high potency (IC_{50} = 1 pM level); it is about 100 times and 30 times more potent than CDDO and dexamethasone, respectively.

These results provide the following SAR about substituents at C-17.

(1) A nitrile group enhances potency. Dinitrile **4** is much more potent than **1** and **2**, nitrile **5** is more potent than **3**.

(2) Ester moieties decrease potency. The less polar the ester, the less is its potency. Ester **12** is much less potent than **1** and **2**.

(3) Tetra-*O*-acetyl-D-glucopyranoside **13** is more potent than **1** and **2**. D-Glucopyranoside **14** is much less potent than **1**, **2**, and **13**. Interestingly, in this case, the more polar the compound, the less is its potency. However, because we have only one example, we cannot conclude that this will be a general relationship.

(4) Amide moieties decrease potency, although amide **15** and hydrazide **16** show similar potency to those of **1** and **2**. The less polar the amide, the less is its potency.

(5) Although carbonyl imidazole **28** is about 30 times more potent than **1**, because this moiety is much more reactive than the other moieties with nucleophiles, it is difficult to compare it with the other moieties. Interestingly, the carbonyl pyrazole **29**, with less reactivity than **28**, is much less potent than **1** and **28**.

Some of these compounds including **4** had good in vivo antiinflammatory activity, when given i.p. or p.o., against peritoneal inflammation induced by thioglycollate and IFN-γ. We will report these data elsewhere. Further biological evaluation of dinitrile **4** is also in progress.

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16. All new compounds **4–29** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses. Dinitrile **4**: amorphous solid; $[\alpha]^{25}_{D}$ +21 ° (*c* 0.29, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 244 (4.30) nm; IR (KBr) 2947, 2871, 2253, 2233, 1690, 1666 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (1H, s), 6.01 (1H, s), 3.26 (1H, d, *J* = 4.8 Hz), 2.78 (1H, ddd, J = 3.3, 4.8, 13.5 Hz), 1.55, 1.53, 1.26, 1.18, 1.01, 1.00, 0.91 (each 3H, s); ^{13}C NMR ($CDCl_3$) δ 197.9, 196.6, 169.4, 165.6, 125.0, 123.9, 114.9, 114.5, 50.1, 47.9, 46.1, 45.2, 42.8, 42.3, 38.4, 35.1, 34.2, 33.7, 33.3, 32.5, 31.9, 30.7, 28.2, 27.2, 26.9, 25.2, 23.9, 23.1, 21.8, 21.7, 18.4; EIMS (70 eV) *m/z* 491 $[M]^+$ (100), 472 (29), 457 (14), 269 (100). HREIMS Calcd for $C_{31}H_{40}N_2O_2$: 472.3090; Found: 472.3095. Anal. Calcd for $C_{31}H_{40}N_2O_2 \cdot H_2O$ C, 75.88; H, 8.63; N, 5.71. Found: C, 75.53; H, 8.58; N, 5.69.

17. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days previously with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 4 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 18.

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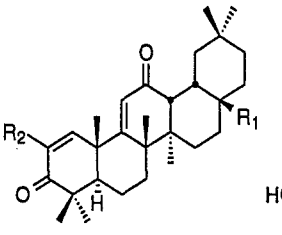
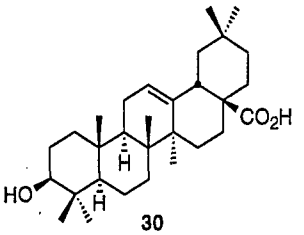
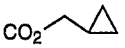
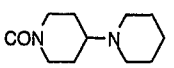
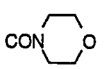
Table 1. Synthesis and Biological Potency of New Oleanane Triterpenoids





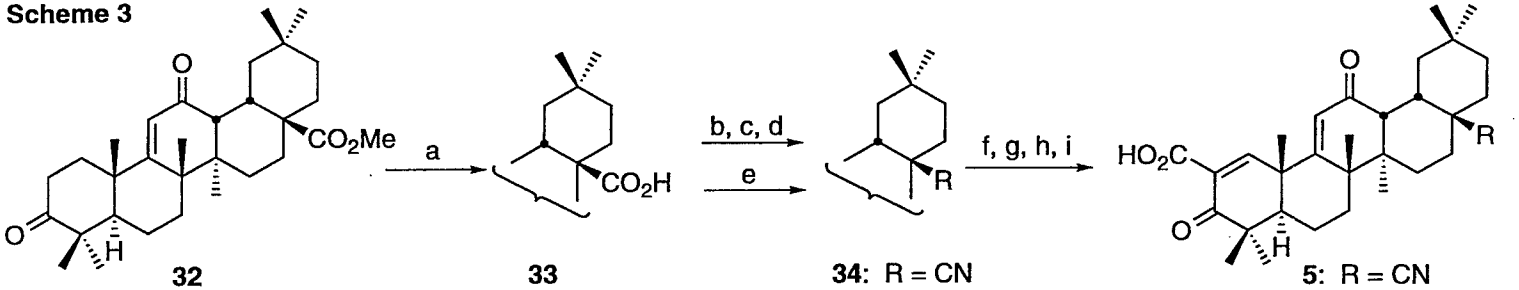
30

compd	R_1	R_2	method	yield (%) from **1**	IC_{50} (nM)[a]
CDDO (**1**)	CO_2H	CN	ref 1 and 4		0.44
2	CO_2Me	CN	ref 1 and 4		0.11
3	CO_2Me	CO_2H	ref 2 and 4		9.55
4	CN	CN	Scheme 1	81	0.0035
5	CN	CO_2H	Scheme 3		1.68
6	CO_2Et	CN	A	100	0.80
7	CO_2Et	CO_2H	Scheme 3		7.93
8	$CO_2CH_2CH{=}CH_2$	CN	B	83	1.33
9	$CO_2(CH_2)_3CH_3$	CN	A	74	6.65
10		CN	A	81	4.45
11	CO_2CH_2Ph	CN	A	97	4.35
12	$CO_2(CH_2)_7CH_3$	CN	A	89	60.4
13	CO-D-Glu$(OAc)_4$	CN	Scheme 2	75	0.070
14	CO-D-Glu	CN	Scheme 2	62	10.1
15	$CONH_2$	CN	Scheme 1	91	0.098
16	$CONHNH_2$	CN	C	55	0.26
17	CONHMe	CN	D	93	0.58
18	$CONH(CH_2)_2CH_3$	CN	D	93	1.50
19	$CONH(CH_2)_5CH_3$	CN	D	92	14.9
20	CONHPh	CN	D	100	28.6
21	$CONHCH_2Ph$	CN	D	96	9.2
22	$CONMe_2$	CN	D	89	1.55
23	CON(*n* -Pr)$_2$	CN	D	85	32.9
24		CN	E	86	0.80
25		CN	E	66	0.95
26		CN	E	82	1.00
27		CN	E	59	2.40
28		CN	C	83	0.014
29		CN	C	92	12.0
30	oleanolic acid				> 40,000
	dexamethasone				0.10

[a] IC_{50} values of compounds **1–29** and dexamethasone were determined in the range of 0.01 pM–1 μM (10-fold dilutions). Values are an average of several separate experiments. None of the compounds were toxic to primary mouse macrophages at 1 μM.

"Reduce to 70% of current size for publication"

Scheme 3

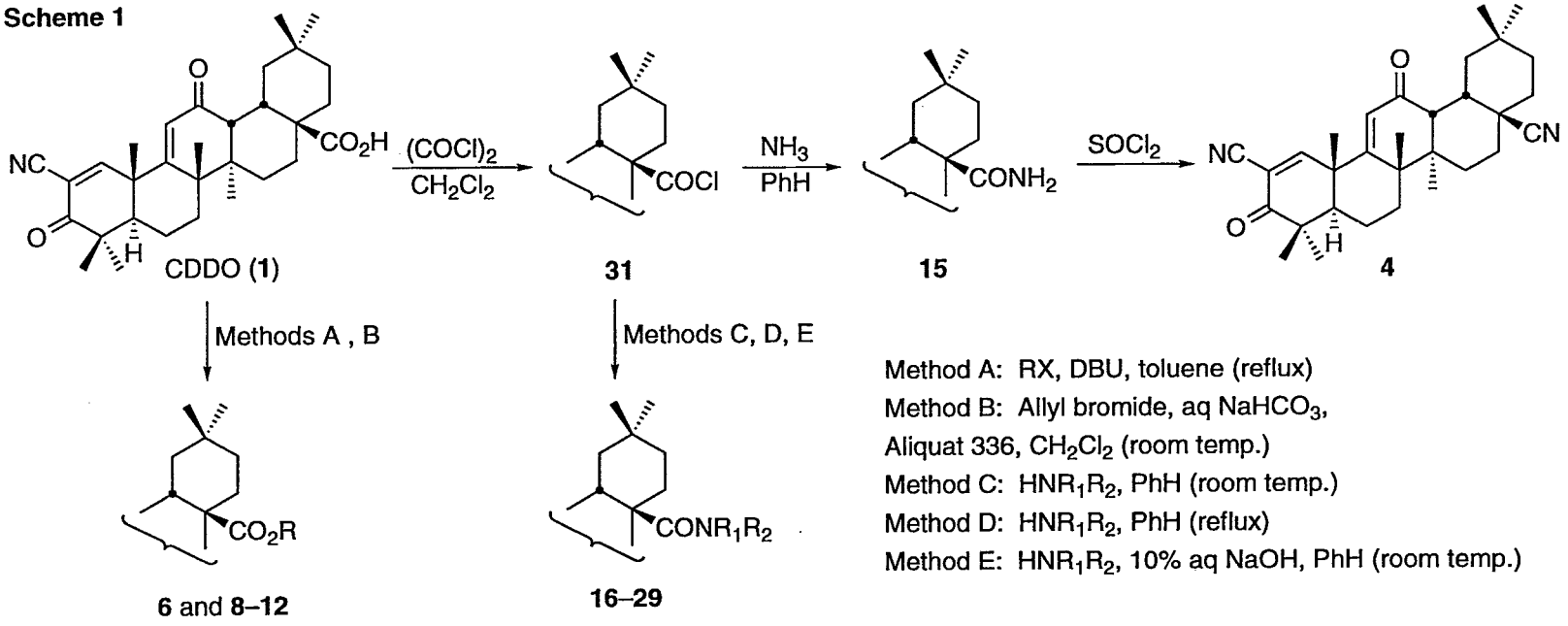


32 **33** **34**: R = CN; **35**: R = CO_2Et **5**: R = CN; **7**: R = CO_2Et

(a) LiI, DMF; (b) $(COCl)_2$, CH_2Cl_2; (c) NH_3, PhH; (d) $SOCl_2$; (e) EtI, DBU, toluene; (f) Stiles' reagent, DMF; (g) CH_2N_2, Et_2O, THF; (h) PhSeCl, pyr., CH_2Cl_2; 30% H_2O_2, CH_2Cl_2; (i) KOH, aq MeOH.

"Reduce to 70% of current size for publication"

Scheme 1



Method A: RX, DBU, toluene (reflux)
Method B: Allyl bromide, aq $NaHCO_3$,
Aliquat 336, CH_2Cl_2 (room temp.)
Method C: HNR_1R_2, PhH (room temp.)
Method D: HNR_1R_2, PhH (reflux)
Method E: HNR_1R_2, 10% aq NaOH, PhH (room temp.)

Scheme 2